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**FIGURE 1**

SEQ ID NO: 4

TTGGAGTGCATTAAGATGCTTAATTATACAGGGTTAGAAAATAAAAATG  
5 TATTAGTTGTCGGTTTGGCAAAAAGTGGTTATGAAGCAGCTAAATTATTAAGTA  
AATTAGGTGCGAATGTAAGTGTCAATGATGGAAAAGACTTATCACAAGATGCT  
CATGCAAAAAGATTTAGAATCTATGGGCATTTCTGTTGTAAGTGGAAAGTCATCCA  
TTAACGTTGCTTGATAATAATCCAATAATTGTTAAAAATCCTGGAATACCTTAT  
ACAGTATCTATTATTGATGAAGCAGTGAAACGAGGTTTGAAAATTTTAACAGA  
10 AGTTGAGTTAAGTTATCTAATCTCTGAAGCACCAATCATAGCTGTAACGGGTAC  
AAATGGTAAAACGACAGTTACTTCTCTAATTGGAGATATGTTTAAAAAAAGTCG  
CTTAACTGGAAGATTATCCGGCAATATTGGTTATGTTGCATCTAAAGTAGCACA  
AGAAGTAAAGCCTACAGATTATTTAGTTACAGAGTTGTCGTCATTCCAGTTACT  
TGGAATCGAAAAGTATAAACCACACATTGCTATAATTACTAACATTTATTCGGC  
15 GCATCTAGATTACCATGAAAATTTAGAAAAGTATCAAAATGCTAAAAAGCAAA  
TATATAAAAATCAAACGGAAGAGGATTATTTGATTTGTAATTATCATCAAAGAC  
AAGTGATAGAGTCGGAAGAATTAAGCTAAGACATTGTATTTCTCAACTCAA  
CAAGAAGTTGATGGTATTTATATTAAAGATGGTTTTATCGTTTATAAAGGTGTT  
CGTATTATTAACACTGAAGATCTAGTATTGCCTGGTGAACATAATTTAGAAAAT  
20 ATATTAGCAGCTGTGCTTGCTTGTATTTTAGCTGGTGTACCTATTAAAGCAATTA  
TTGATAGTTTAACTACATTTTCAGGAATAGAGCATAGATTGCAATATGTTGGTA  
CTAATAGAACTAATAAATATTATAATGATTCCAAAGCAACAAACACGCTAGCA  
ACACAGTTTGCCTTAAATTCATTTAATCAACCAATCATTGTTATGTGGTGGTT  
TGGATCGAGGGAATGAATTTGACGAACTCATTCCTTATATGGAAAATGTTTCGCG  
25 CGATGGTTGTATTCGGACAAACGAAAGCTAAGTTTGCTAAACTAGGTAATAGTC  
AAGGGAAATCGGTCATTGAAGCGAACAATGTCGAAGACGCTGTTGATAAAGTA  
CAAGATATTATAGAACCAAATGATGTTGTATTATTGTCACCTGCTTGTGCGAGT  
TGGGATCAATATAGTACTTTTGAAGAGCGTGGAGAGAAATTTATTGAAAGATTC  
CGTGCCCATTTACCATCTTATTAA

**FIGURE 2**

SEQ ID NO: 5

LECIKMLNYTGLENKNVLVVGLAKSGYEAAKLLSKLGANVTVNNDGKDLSQ  
5 DAHAKDLESMGISVVSGSHPLTLLDNNPIIVKNPGIPYTVSIIDEAVKRGLKILTEVE  
LSYLISEAPIIAVTGTNGKTTVTSLIGDMFKKSRLTGRLSGNIGYVASKVAQEVKPT  
DYLVTTELSSFQLLGIEKYKPHIAIITNIYSAHLDYHENLENYQNAKKQIYKNQTEED  
YLICNYHQRQVIESEELKAKTLYFSTQQEVDGIYIKDGFIVYKGVRIINTEDLVLPGE  
HNLENILAAVLACILAGVPIKAIIDSLTTFSGIEHRLQYVGTNRTNKKYYNDSKATNTL  
10 ATQFALNSFNQPIIWLCGGLDRGNEFDELIPYMENVRAMVVFGQTKAKFAKLGNS  
QGKSVIEANNVEDAVDKVQDIIEPNDVLLSPACASWDQYSTFEERGEKFIERFRA  
HLPSY

**FIGURE 3**

SEQ ID NO: 6

TTGGAGTGCATTAAGATGCTTAATTATACAGGGTTAGAAAATAAAAATA  
5 TATTAGTTGTCGGTTTGGCAAAAAGTGGTTATGAAGCAGCTAAATTATTAAGTA  
AATTAGGTGCGAATGTAAGTGTCAATGATGGAAAAGACTTATCACAAGATGCT  
CATGCAAAAAGATTTAGAATCTATGGGCATTTCTGTTGTAAGTGGAAAGTCATCCA  
TTAACGTTGCTTGATAATAATCCAATAATTGTTAAAAATCCTGGAATACCTTAT  
ACAGTATCTATTATTGATGAAGCAGTGAAACGAGGTTTGAAAATTTTAACAGA  
10 AGTTGAGTTAAGTTATCTAATCTCTGAAGCACCAATCATAGCTGTAACGGGTAC  
AAATGGTAAAACGACAGTTACTTCTCTAATTGGAGATATGTTTAAAAAAAGTCG  
CTTAACTGGAAGATTATCCGGCAATATTGGTTATGTTGCATCTAAAGTAGCACA  
AGAAGTAAAGCCTACAGATTATTTAGTTACAGAGTTGTCGTCATTCCAGTTACT  
TGGAATCGAAAAGTATAAACCACACATTGCTATAATTACTAACATTTATTCGGC  
15 GCATCTAGATTACCATGAAAATTTAGAAAAGTATCAAAATGCTAAAAAGCAAA  
TATATAAAAATCAAACGGAAGAGGATTATTTGATTTGTAATTATCATCAAAGAC  
AAGTGATAGAGTCGGAAGAATTAAAAGCTAAGACATTGTATTTCTCAACTCAA  
CAAGAAGTTGATGGTATTTATATTAAAGATGGTTTTATCGTTTATAAAGGTGTT  
CGTATTATTAACACTGAAGATCTAGTATTGCCTGGTGAACATAATTTAGAAAAT  
20 ATATTAGCAGCTGTGCTTGCTTGTATTTTAGCTGGTGTACCTATTAAAGCAATTA  
TTGATAGTTTAACTACATTTTCAGGAATAGAGCATAGATTGCAATATGTTGGTA  
CTAATAGAACTAATAAATATTATAATGATTCCAAAGCAACAAACACGCTAGCA  
ACACAGTTTGCCTTAAATTCATTTAATCAACCAATCATTGTTGTTATGTGGTGGTT  
TGGATCGAGGGAATGAATTTGACGAACTCATTCCTTATATGGAAAATGTTTCGCG  
25 TGATGGTTGTATTCGGACAAACGAAAGCTAAGTTTGCTAACTAGGTAATAGTC  
AAGGGAAATCGGTCATTGAAGCGAACAATGTCGAAGACGCTGTTGATAAAGTA  
CAAGATATTATAGAACCAAATGATGTTGTATTATTGTCACCTGCTTGTGCGAGT  
TGGGATCAATATAGTACTTTTGAAGAGCGTGGAGAGAAATTTATTGAAAGATTC  
CGTGCCCATTTACCATCTTATTAA



**FIGURE 4**

SEQ ID NO: 7

LECIKMLNYTGLENKNILVVGLAKSGYEAAKLLSKLGANVTVNDGKDLSQ  
5 DAHAKDLESMGISVVSGSHPLTLLDNNPIIVKNPGIPYTVSIIDEAVKRGLKILTEVE  
LSYLISEAPIIAVTGTNGKTTVTSLIGDMFKKSRLTGRLSGNIGYVASKVAQEVKPT  
DYLVTLSFQLLGIEKYKPHIAIITNIYSAHLDYHENLENYQNAKKQIYKNQTEED  
YLICNYHQRQVIESEELKAKTLYFSTQQEVDGIYIKDGFIVYKGVRIINTEDLVLPGE  
HNLENILAAVLACILAGVPIKAIDSLTTFSGIEHRLQYVGTNRTNKKYYNDSKATNTL  
10 ATQFALNSFNQPIIWLCGGLDRGNEFDELIPYMENVRVMVVFGQTKAKFAKLGNS  
QGKSVIEANNVEDAVDKVQDIIEPNDVLLSPACASWDQYSTFEERGEKFIERFRA  
HLPSY

**FIGURE 5**

SEQ ID NO: 8

Forward PCR Primer

5 GCGGCGGCCCATATGCCAATTATTACAGATGTTTAC

10 SEQ ID NO: 9

Reverse PCR Primer

GCGCGGATCCTTATGAAAATTCACCTTCAATAATTTC

15

**FIGURE 6****TABLE 1 Properties of UDP-N-acetylmuramoylalanine-D-glutamate ligase from *S. aureus***

TABLE 1 -- UDP-N-acetylmuramoylalanine-D-glutamate ligase from <i>S. aureus</i> -- SEQ ID NO: 4-SEQ ID NO: 7	
Melting temperature (°C) of SEQ ID NO: 8 (forward PCR primer)	62
Restriction enzyme for SEQ ID NO: 8 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 9 (reverse PCR primer)	60
Restriction enzyme for SEQ ID NO: 9 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 4	1365
Number of amino acid residues in SEQ ID NO: 5	454
Number of different nucleic acid residues between SEQ ID NO: 4 and SEQ ID NO: 6	2
Number of different amino acid residues between SEQ ID NO: 5 and SEQ ID NO: 7	2
Calculated molecular weight of SEQ ID NO: 5 polypeptide (kDa)	50.5
Calculated pI of SEQ ID NO: 5 polypeptide	5.3
Solubility of SEQ ID NO: 7 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching 100%
Amount of purified polypeptide having SEQ ID NO: 7, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	16.3
Amount of purified polypeptide having SEQ ID NO: 7 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	29.6
Z-score for the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 7, determined as described in EXAMPLE 9	8.0E-08
Number of matched peptides in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 7, determined as described in EXAMPLE 9	16
Minimum sequence coverage in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 7, determined as described in EXAMPLE 9	42
Calculated molecular weight of SEQ ID NO: 5 polypeptide (Da), determined as described in EXAMPLE 10	52460
Experimental molecular weight of SEQ ID NO: 7 polypeptide (Da), determined as described in EXAMPLE 10	50772
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. The identity of an interacting protein identified by using at least one of the methods described in those examples is: 90 kDa unidentified protein.	

FIGURE 6-B

TABLE 1 continued: Truncation Polypeptides of UDP-N-acetylmuramoylalanine-D-glutamate ligase from *S. aureus*

UDP-N-acetylmuramoylalanine-D-glutamate ligase from <i>S. aureus</i> -- SEQ ID NO: 4-SEQ ID NO: 7					
Start of truncated polypeptide of SEQ ID NO: 7	T5	L7	N9	Y4	
End of truncated polypeptide of SEQ ID NO: 7	I439	R443	H445	L446	
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one-third	Approaching one-third	Approaching one-third	Approximately two-thirds	
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	No discernable expression			No discernable expression	
Amount of purified truncated polypeptide, prepared and purified as described in the Exemplification (mg/L of culture).	5.0 (1)	5.0 (1)	15.0 (1)	19.0	
Amount of purified, truncated polypeptide soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	30.0	19.0	46.0	31.0	
Z-score for the peptide fingerprint mapping analysis of truncated polypeptide, determined as described in EXAMPLE 9	5.10E-03		3.6E-09	2.4E-08	
Number of matched peptides in the peptide fingerprint mapping analysis of truncated polypeptide, determined as described in EXAMPLE 9	8		19	21	
Minimum sequence coverage in the peptide fingerprint mapping analysis of truncated polypeptide, determined as described in EXAMPLE 9	26%		50%	57%	
Calculated molecular weight of truncated polypeptide (Da), determined as described in EXAMPLE 10	50225		50622	51299	
Experimental molecular weight of truncated polypeptide (Da), determined as described in EXAMPLE 10	50402		50734	51466	

(1) The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.

**FIGURE 6-C****TABLE 1 continued: Truncation Polypeptides of UDP-N-acetylmuramoylalanine-D-glutamate ligase from *S. aureus***

UDP-N-acetylmuramoylalanine-D-glutamate ligase from <i>S. aureus</i> -- SEQ ID NO: 4-SEQ ID NO: 7				
Start of truncated polypeptide of SEQ ID NO: 7	N3	N3	N3	N3
End of truncated polypeptide of SEQ ID NO: 7	I439	R441	R443	H445
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Less than one-third	No discernable expression	No discernable expression	No discernable expression
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)		No discernable expression		

UDP-N-acetylmuramoylalanine-D-glutamate ligase from <i>S. aureus</i> -- SEQ ID NO: 4-SEQ ID NO: 7				
Start of truncated polypeptide of SEQ ID NO: 7	T5	T5	T5	L7
End of truncated polypeptide of SEQ ID NO: 7	R441	R443	H445	I439
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	No discernable expression	No discernable expression	No discernable expression	No discernable expression
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)			No discernable expression	No discernable expression

UDP-N-acetylmuramoylalanine-D-glutamate ligase from <i>S. aureus</i> -- SEQ ID NO: 4-SEQ ID NO: 7				
Start of truncated polypeptide of SEQ ID NO: 7	L7	N9	N9	M1
End of truncated polypeptide of SEQ ID NO: 7	R441	I439	R441	R443
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	No discernable expression	No discernable expression	No discernable expression	Less than one-third
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	Approaching one-third		No discernable expression	No discernable expression

FIGURE 6-D

TABLE 1 continued: Truncation Polypeptides of UDP-N-acetylmuramoylalanine-D-glutamate ligase from *S. aureus*

UDP-N-acetylmuramoylalanine-D-glutamate ligase from <i>S. aureus</i> -- SEQ ID NO: 4-SEQ ID NO: 7					
Start of truncated polypeptide of SEQ ID NO: 7	N11	N11	N11	K10	G16
End of truncated polypeptide of SEQ ID NO: 7	R441	R443	H445	I439	L446
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one-third	Approaching one-third	No discernable expression	No discernable expression	No discernable expression
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)				No discernable expression	No discernable expression

PCR primers and restriction enzymes used to prepare the truncated polypeptides of TABLE 1, and the deleted amino acid residues in them, are set forth in the following tables:

Start of truncated polypeptide	N3	T5	L7	N9	N11
Residues deleted from N-terminus	ME	MECI	MECIKM	MECIKMLN	MECIKMLNYT
Nucleic acid sequence of forward PCR primer	SEQ ID NO: 10 G CGCGGGCCCA TATGAATTATA CAGGGTTAGA AAATAAAAAT G	SEQ ID NO: 11 G CGCGGGCCCA TATGACAGGG TTAGAAAATA AAAATG	SEQ ID NO: 12 G CGCGGGCCCA TATGTTAGAA AATAAAAATG TATTAG	SEQ ID NO: 13 G CGCGGGCCCA TATGAATAAA AATGTATTAGT TGTC	SEQ ID NO: 14 G CGCGGGCCCA TATGAATGTAT TAGTTGTCGGT TTG
Restriction enzyme for forward PCR primer	NdeI	NdeI	NdeI	NdeI	NdeI

**FIGURE 6-E****TABLE 1 continued: Truncation Polypeptides of UDP-N-acetylmuramoylalanine-D-glutamate ligase from *S. aureus***

PCR primers and restriction enzymes used to prepare the truncated polypeptides of TABLE 1, and the deleted amino acid residues in them, are set forth in the following tables:

Start of truncated polypeptide	Y4	K10	G16
Residues deleted from N-terminus	MEC	MECIKMLNY	MECIKMLNYTGLENK
Nucleic acid sequence of forward PCR primer	SEQ ID NO: 15 GCGGCG	SEQ ID NO: 16 GCGGCG	SEQ ID NO: 17 GCGGCG
	GCCCATATGTATACAG	GCCCATATGAAAAATG	GCCCATATGGGTTTGG
	GGTTAGAAAAATAAAA ATG	TATTAGTTGTCGG	CAAAAAAGTGG
Restriction enzyme for forward PCR primer	NdeI	NdeI	NdeI

End of truncated polypeptide	I439	R441	R443
Residues deleted from C-terminus	GEKFIERFRAHLPSY	KFIERFRAHLPSY	IERFRAHLPSY
Nucleic acid sequence of reverse PCR primer	SEQ ID NO: 18 GCGCGG	SEQ ID NO: 19 GCGCGG	SEQ ID NO: 20 GCGCGG
	ATCCAATAAAATTTCTCT	ATCCTCTTTCAATAAAT	ATCCACGGAATCTTTCA
	CCACGCTC	TTCTCTCCAC	ATAAATTTCTC
Restriction enzyme for reverse PCR primer	BamHI	BamHI	BamHI

End of truncated polypeptide	H445	L446	Y449
Residues deleted from C-terminus	RFRAHLPSY	FRAHLPSY	HLPSY
Nucleic acid sequence of reverse PCR primer	SEQ ID NO: 21 GCGCGG	SEQ ID NO: 22 GCGCGG	SEQ ID NO: 23 GCGCGG
	ATCCATGGGCACGGAA	ATCCTAAATGGGCACG	ATCCATAAGATGGTAA
	TCTTTCAATAAAATTC	GAATCTTTC	ATGGGCACG
Restriction enzyme for reverse PCR primer	BamHI	BamHI	BamHI

A blank in any of the parts of TABLE 1 indicates that the experiment was not completed.

## FIGURE 7

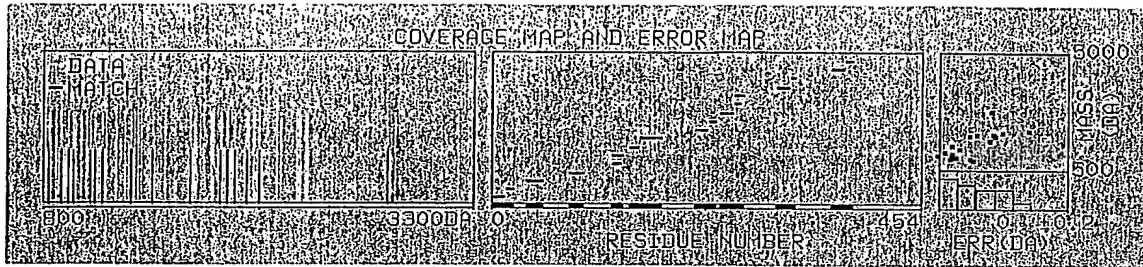
**TABLE 2 Bioinformatic Analyses of UDP-N-acetylmuramoylalanine-D-glutamate ligase from *S. aureus***

TABLE 2 -- UDP-N-acetylmuramoylalanine-D-glutamate ligase from <i>S. aureus</i> -- SEQ ID NO: 4-SEQ ID NO: 7	
COG Category	Cell envelope biogenesis, outer membrane
COG ID Number	COG0771
Is SEQ ID NO: 5 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 5	UDP-N-Acetylmuramoyl-L-Alanine, (1eeh_A)
Source organism for closest PDB protein to SEQ ID NO: 5	<i>Escherichia coli</i>
e-value for closest PDB Protein to SEQ ID NO: 5	3E-48
% Identity between SEQ ID NO: 5 and the closest protein from PDB	30
% Positives between SEQ ID NO: 5 and the closest protein from PDB	49
Number of Protein Hits in the VGDB to SEQ ID NO: 5	9
Number of Microorganisms having VGDB Hits to SEQ ID NO: 5	9
Microorganisms having VGDB Hits to SEQ ID NO: 5 <sup>1</sup>	[saur][bsub][spne][efae][hinf][nmen][paer][rpxx][bbur]
First predicted epitopic region of SEQ ID NO: 5: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 24 :KNVLVVGLAKS, 1.227,15->25
Second predicted epitopic region of SEQ ID NO: 5: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 25 :ENILAAVLACILAG-VPIKAIDSLTTF, 1.225,284->310
Third predicted epitopic region of SEQ ID NO: 5: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 26 :PNDVVLLSPACASWD, 1.205,417->431

- 5                   <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Escherichia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpxx* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.



FIGURE 8



Note: click on the ☐ symbol to change column format.

Measured	Avg/	Computed	Error	<input type="checkbox"/> Residues	Missed		
Mass (M)	Mono	Mass	(Da)	Start	To	Cut	Peptide sequence
840.348	M	840.438	-0.090	259	265	0	DGFIVYK
911.423	M	911.580	-0.157	16	24	0	NVLVVGLAK
949.399	M	949.498	-0.098	317	324	0	LQYVGTR
979.375	M	979.516	-0.140	376	384	0	AMVVFQTK
1073.367	M	1073.560	-0.193	232	240	0	QVIESEELK
1107.438	M	1107.592	-0.154	147	157	0	LSGNIGYVASK
1152.455	M	1152.629	-0.174	259	268	1	DGFIVYKGV
1311.541	M	1311.674	-0.133	128	139	0	TTVTSIGDMFK
1439.617	M	1439.769	-0.151	128	140	1	TTVTSIGDMFKK
1658.822	M	1658.862	-0.040	302	316	0	AIIDSLTTFSGIEHR
1824.811	M	1824.910	-0.100	1	15	1	LECIKMLNYTGLENK
1824.811	M	1824.834	-0.024	361	375	0	GNEFDELIPYMEVNR
1870.987	M	1871.014	-0.027	83	99	1	NPGIPYTVSIIDEAVKR
1903.879	M	1903.956	-0.077	243	258	0	TLVFSTQQEVDGIYIK
1981.855	M	1981.858	-0.003	217	231	0	NQTEEDYLICNYHQR
2052.103	M	2052.023	0.080	36	55	1	LGANVTVNDGKDLSDAHAK
2806.463	M	2806.499	-0.036	158	182	0	VQEVKPTDYLVTLSFQLLGIEK

FIGURE 9

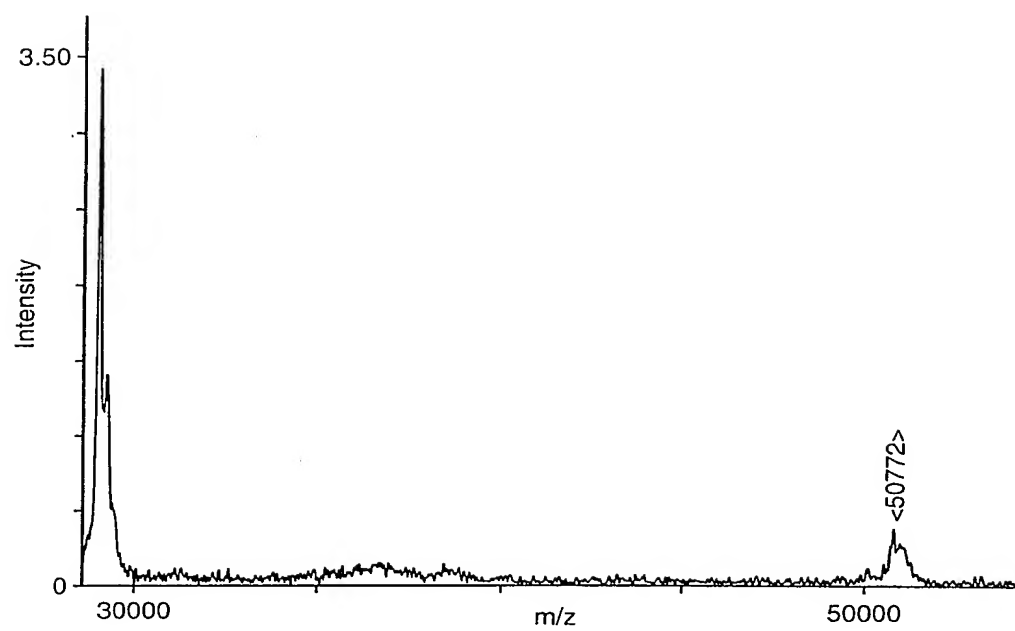


FIGURE 10



Note: click on the ☐ symbol to change column format.

Measured	Avg/	Computed	Error	<input type="checkbox"/> Residues	Missed		
Mass (E)	Mono	Mass	(Da)	Start	To	Cut	Peptide sequence
840.455	H	840.438	0.017	259	265	0	DGFIVYK
949.466	H	949.498	-0.032	317	324	0	LQYVGTNR
1439.729	H	1439.769	-0.040	128	140	1	TTVTSIGDMFKK
1658.850	H	1658.862	-0.011	302	316	0	AIIDSLTFSGIEHR
1824.809	H	1824.910	-0.102	1	15	1	LECIKMLNYTGLENK
1824.809	H	1824.834	-0.026	361	375	0	GNEFDELIPYMEIVR
1870.988	H	1871.014	-0.026	83	99	1	NPGIPYTVSIIDEAVKR
1903.910	H	1903.956	-0.046	243	258	0	TLVFSTQQEVDGIYIK
2806.654	H	2806.499	0.155	158	182	0	VAQEVKPTDYLVTLSFQLLGIEK

FIGURE 11

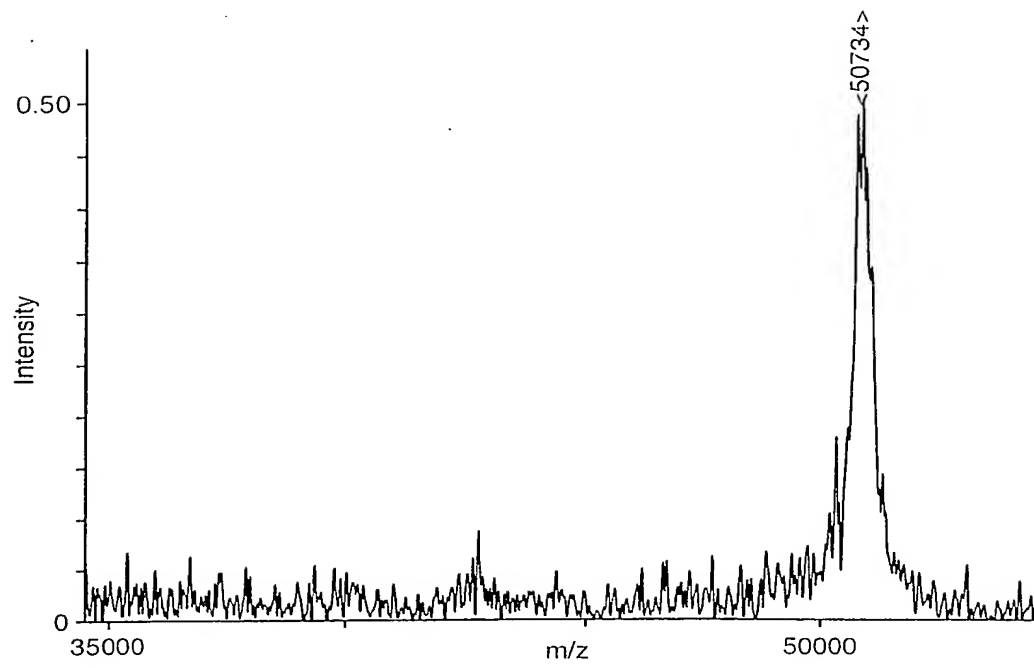
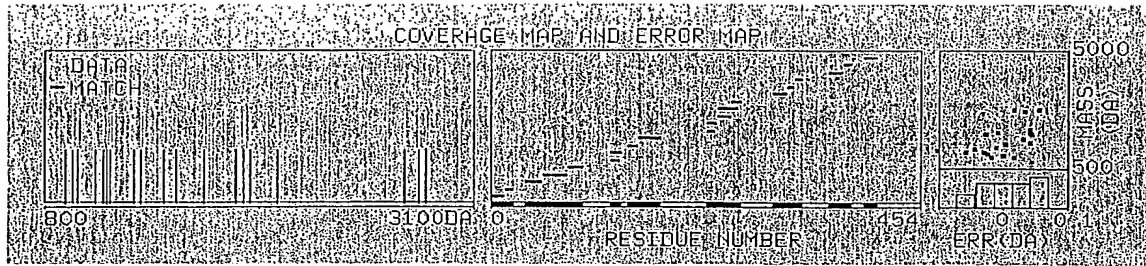


FIGURE 12



Note: click on the ☐ symbol to change column format.

Measured Mass (M)	Avg/ Mono	Computed Mass	Error (Da)	<input checked="" type="checkbox"/> Residues	Missed	Peptide sequence
				Start	To	
911.561	M	911.580	-0.019	16	24	0 NVLVVG LAK
949.518	M	949.498	0.020	317	324	0 LQYVGTNR
979.517	M	979.516	0.001	376	384	0 ANVVFQTK
1073.536	M	1073.560	-0.024	232	240	0 QVIESEELK
1107.534	M	1107.592	-0.058	147	157	0 LSGNIGYVASK
1131.489	M	1131.519	-0.030	325	333	1 TNKYND SK
1152.526	M	1152.629	-0.103	259	268	1 DGFIVYK GVR
1272.647	M	1272.692	-0.045	232	242	1 QVIESEELKAK
1311.664	M	1311.674	-0.009	128	139	0 TTVTSLIGDMFK
1439.773	M	1439.769	0.005	128	140	1 TTVTSLIGDMFKK
1501.771	M	1501.725	0.045	397	410	0 SVIEANINVEDAVDK
1658.895	M	1658.862	0.033	302	316	0 AIIDSLTTFSGIEHR
1824.882	M	1824.910	-0.028	1	15	1 LECIKHLNPTGLENK
1824.882	M	1824.834	0.048	361	375	0 GNEFDELIPYHENVK
1871.060	M	1871.014	0.045	83	99	1 NPGIPYTVSIIDEAVKR
1903.950	M	1903.956	-0.006	243	258	0 TLYFSTQQEV DGIYK
2052.068	M	2052.023	0.045	36	55	1 LGANVTVNDGKDL SQDAHAK
2726.420	M	2726.383	0.037	243	265	1 TLYFSTQQEV DGIYK DGFIVYK
2806.517	M	2806.499	0.018	158	182	0 VAQEVKPTDYLVT ELSSFQLLGIEK
2847.562	M	2847.504	0.058	56	82	0 DLESHGISVVS GSHPLTLLDNNPIIVK

FIGURE 13

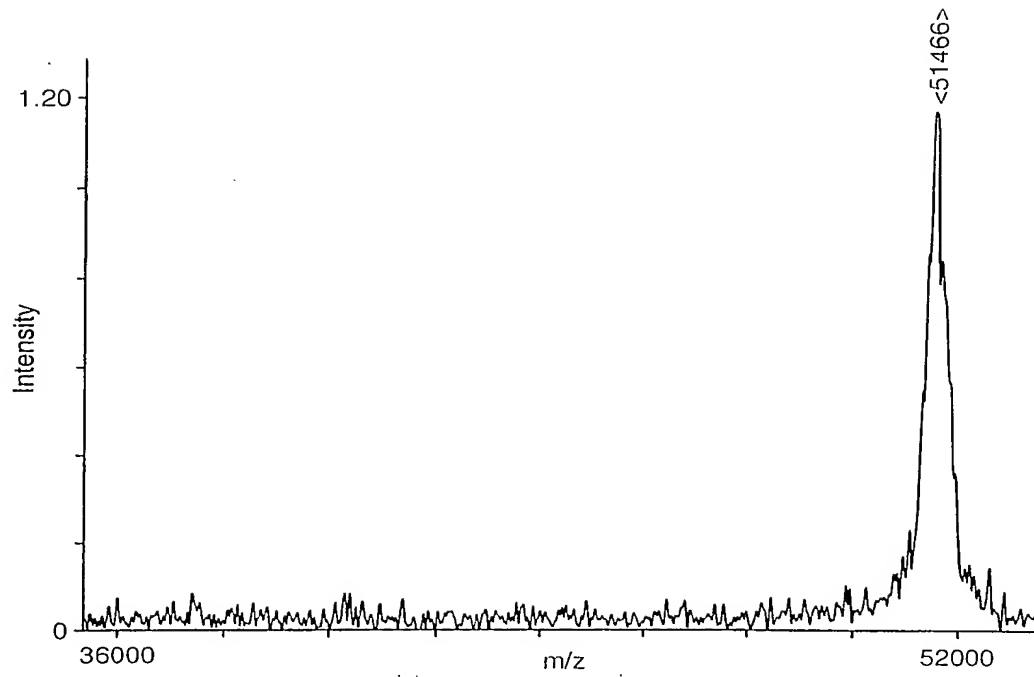
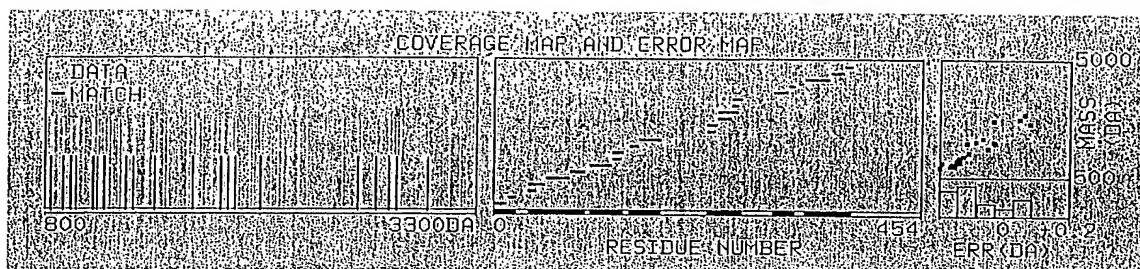


FIGURE 14



Note: click on the ☐ symbol to change column format.

Measured	Avg/	Computed	Error	<input type="checkbox"/> Residues	Missed		
Mass (M)	Mono	Mass	(Da)	Start	To	Cut	Peptide sequence
840.261	M	840.438	-0.176	259	265	0	DGFIVYK
911.445	M	911.580	-0.134	16	24	0	NVLVVGLAK
949.354	M	949.498	-0.144	317	324	0	LQYVGTHR
979.376	M	979.516	-0.140	376	384	0	AMVVFQTK
1073.438	M	1073.560	-0.122	232	240	0	QVIESEELK
1086.397	M	1086.566	-0.169	36	46	0	LGANVTVNDGK
1107.461	M	1107.592	-0.131	147	157	0	LSGHIGYVASK
1152.504	M	1152.629	-0.125	259	268	1	DGFIVYKGVK
1272.569	M	1272.692	-0.122	232	242	1	QVIESEELKAK
1311.564	M	1311.674	-0.110	128	139	0	TTVTSLIGDMFK
1439.674	M	1439.769	-0.094	128	140	1	TTVTSLIGDMFKK
1658.821	M	1658.862	-0.041	302	316	0	AIIDSLTFSGIEHR
1824.810	M	1824.910	-0.100	1	15	1	LECIKMLNYTGLENK
1824.810	M	1824.834	-0.024	361	375	0	GNEFDELIPYMNVR
1870.988	M	1871.014	-0.027	83	99	1	NPGIPYTVSIIDEAVKR
1903.879	M	1903.956	-0.077	243	258	0	TLYFSTQQEVVDGIYK
2051.968	M	2052.023	-0.055	36	55	1	LGANVTVNDGKDLSDAHAK
2630.513	M	2630.440	0.073	103	127	0	ILTEVELSYLISEAPIIAVTGTNGK
2726.358	M	2726.383	-0.025	243	265	1	TLYFSTQQEVVDGIYKDGFIYK
2806.539	M	2806.499	0.040	158	182	0	VAQEVKPTDYLVTLSFQLLGIEK
2847.544	M	2847.504	0.040	56	82	0	DLESMGISVVGSHPLTLDDNNPIIVK
3020.572	M	3020.516	0.055	334	360	0	ATNTLATQFALNSFNQPIIWLCCGLDR

**FIGURE 15**

SEQ ID NO: 27

ATGAGTAAGGAGTTTTATATAATGACACACTATCATTTTTGTCGGAATTAA  
5 AGGTTCTGGCATGAGTTCATTAGCACAAATCATGCATGATTTAGGACATGAAGT  
TCAAGGATCGGATATTGAGAACTACGTATTTACAGAAGTTGCTCTTAGAAATAA  
GGGGATAAAAATATTACCATTTGATGCTAATAACATAAAAGAAGATATGGTAG  
TTATACAAGGTAATGCATTCGCGAGTAGCCATGAAGAAATAGTACGTGCACAT  
CAATTGAAATTAGATGTTGTAAGTTATAATGATTTTTTAGGACAGATTATTGAT  
10 CAATATACTTCAGTAGCTGTAAGTGGTGCACATGGTAAACTTCTACAACAGGT  
TTATTATCACATGTTATGAATGGTGATAAAAAGACTTCATTTTTAATTGGTGAT  
GGCACAGGTATGGGATTGCCTGAAAGTGATTATTTTCGCTTTTGAGGCATGTGAA  
TATAGACGTCACTTTTTAAGTTATAAACCTGATTACGCAATTATGACAAATATT  
GATTTTCGATCATCCTGATTATTTTAAAGATATTAATGATGTTTTTGATGCATTCC  
15 AAGAAATGGCACATAATGTTAAAAAAGGTATTATTGCTTGGGGTGATGATGAA  
CATCTACGTAAAATTGAAGCAGATGTTCCAATTTATTATTATGGATTTAAAGAT  
TCGGATGACATTTATGCTCAAAATATTCAAATTACGGATAAAGGTACTGCTTTT  
GATGTGTATGTGGATGGTGAGTTTTATGATCACTTCCTGTCTCCACAATATGGT  
GACCATACAGTTTTTAAATGCATTAGCTGTAATTGCGATTAGTTATTTAGAGAAG  
20 CTAGATGTTACAAATATTAAAGAAGCATTAGAAACGTTTGGTGGTGTTAAACGT  
CGTTTCAATGAAACTACAATTGCAAATCAAGTTATTGTAGATGATTATGCACAC  
CATCCAAGAGAAATTAGTGCTACAATTGAAACAGCACGAAAGAAATATCCACA  
TAAAGAAGTTGTTGCAGTATTTCAACCACACACTTTCTCTAGAACACAGGCATT  
TTTAAATGAATTTGCAGAAAGTTTAAAGTAAAGCAGATCGTGTATTCTTATGTGA  
25 AATTTTTGGATCAATTAGAGAAAATACTGGCGCATTAAACGATACAAGATTTAAT  
TGATAAAATTGAAGGTGCATCGTTAATTAATGAAGATTCTATTAATGTATTAGA  
ACAATTTGATAATGCTGTTATTTTATTTATGGGTGCAGGTGATATTCAAAAATT  
ACAAAATGCATATTTAGATAAATTAGGCATGAAAAATGCGTTTTAA



**FIGURE 16**

SEQ ID NO: 28

MSKEFYIMTHYHFVGIKGSGMSSLAQIMHDLGHEVQGSDIENYVFTEVALR  
5 NKGILPFDANNIKEDMVVIQGNFASSHEEIVRAHQLKLDVVSYNDFLGQIIDQY  
TSVAVTGAHGKTSTTGLLSHVMNGDKKTSFLIGDGTGMGLPESDYFAFEACEYRR  
HFLSYKPDYAIMTNIDFDHPDYFKDINDVFDAFQEMAHNVKKGIWGDDEHLRKI  
EADVPIYYYGFKDSDDIYAQNIQITDKGTAFDVYVDGEFYDHFLSPQYGDHTVLNA  
LAVIAISYLEKLDVTNIKEALETFGGVKRRFNETTIANQVIVDDYAHHPREISATIET  
10 ARKKYPHKEVVAVFQPHTFSRTQAFLNEFAESLSKADRVFLCEIFGSIRENTGALTI  
QDLIDKIEGASLINEDSINVLEQFDNAVILFMGAGDIQKLQNAVYLDKLGKMKNAF

**FIGURE 17**

SEQ ID NO: 29

ATGAGTAAGGAGTTTTATATAATGACACACTATCATTTTTGTCGGAATTAA  
5 AGGTTCTGGCATGAGTTCATTAGCACAAATCATGCATGATTTAGGACATGAAGT  
TCAAGGATCGGATATTGAGAACTACGTATTTACAGAAGTTGCTCTTAGAAATAA  
GGGGATAAAAATATTACCATTTGATGCTAATAACATAAAAGAAGATATGGTAG  
TTATACAAGGTAATGCATTCGCGAGTAGCCATGAAGAAATAGTACGTGCACAT  
CAATTGAAATTAGATGTTGTAAGTTATAATGATTTTTTAGGACAGATTATTGAT  
10 CAATATACTTCAGTAGCTGTAAGTGGTGCACATGGTAAAACCTTCTACAACAGGT  
TTATTATCACATGTTATGAATGGTGATAAAAAGACTTCATTTTTAATTGGTGAT  
GGCACAGGTATGGGATTGCCTGAAAGTGATTATTTTCGCTTTTGAGGCATGTGAA  
TATAGACGTCACCTTTTAAAGTTATAAACCTGATTACGCAATTATGACAAATATT  
GATTTTCGATCATCCTGATTATTTTAAAGATATTAATGATGTTTTTGATGCATTCC  
15 AAGAAATGGCACATAATGTAAAAAAGGTATTATTGCTTGGGGTGATGATGAA  
CATTTACGTAAAATTGAAGCAGATGTTCCAATTTATTATTATGGATTTAAAGAT  
TCGGATGACATTTATGCTCAAAATATTCAAATTACGGATAAAGGTACTGCTTTT  
GATGTGTATGTGGATGGTGAGTTTTATGATCACTTCCTGTCTCCACAATATGGT  
GACCATACAGTTTTAAATGCATTAGCTGTAATTGCGATTAGTTATTTAGAGAAG  
20 CTAGATGTTACAAATATTAAAGAAGCATTAGAAACGTTTGGTGGTGTTAAACGT  
CGTTTCAATGAAACTACAATTGCAAATCAAGTTATTGTAGATGATTATGCACAC  
CATCCAAGAGAAATTAGTGCTACAATTGAAACAGCACGAAAGAAATATCCACA  
TAAAGAAGTTGTTGCAGTATTTCAACCACACACTTTCTCTAGAACACAGGCATT  
TTAAATGAATTTGCAGAAAGTTTAAAGTAAAGCAGATCGTGTATTCTTATGTGA  
25 AATTTTTGGATCAATTAGAGAAAATACTGGCGCATTAAACGATACAAGATTTAAT  
TGATAAAATTGAAGGTGCATCGTTAATTAATGAAGATTCTATTAATGTATTAGA  
ACAATTTGATAATGCTGTTATTTTATTTATGGGTGCAGGTGATATTCAAAAATT  
ACAAAATGCATATTTAGATAAATTAGGCATGAAAAATGCGTTTTAA

**FIGURE 18**

SEQ ID NO: 30

MSKEFYIMTHYHFVGIKGSGMSSLAQIMHDLGHEVQGSDIENYVFTEVALR  
5 NKGIKILPFDANNIKEDMVVIQGNAFASSHEEIVRAHQLKLDVVSYNDFLGQIIDQY  
TSVAVTGAHGKTSTTGLLSHVMNGDKKTSFLIGDGTGMGLPESDYFAFEACEYRR  
HFLSYKPDYAIMTNIDFDHPDYFKDINDVFDAFQEMAHNVKKGIILAWGDDEHLRKI  
EADVPIYYYGFKDSDDIYAQNIQITDKGTAFDVYVDGEFYDHFLSPQYGDHTVLNA  
LAVIAISYLEKLDVTNIKEALETFGGVKRRFNETTIANQVIVDDYAHHPREISATIET  
10 ARKKYPHKEVVAVFQPHTFSRTQAFLNEFAESLSKADRVFLCEIFGSIRENTGALTI  
QDLIDKIEGASLINEDSINVLEQFDNAVILFMGAGDIQKLQNAVLDKLGMKNAF

**FIGURE 19**

SEQ ID NO: 31

Forward PCR Primer

5 GCGGCGGCCCATATGACAGTATTAACAGATAAAGTAG

SEQ ID NO: 32

10

Reverse PCR Primer

GCGCGGATCCTTAAACAATATCCAAACCACCGAATG

**FIGURE 20****TABLE 3 Properties of UDP-N-acetylmuramate-alanine ligase from *S. aureus***

TABLE 3 -- UDP-N-acetylmuramate-alanine ligase from <i>S. aureus</i> -- SEQ ID NO: 27-SEQ ID NO: 30	
Melting temperature (°C) of SEQ ID NO: 31 (forward PCR primer)	64
Restriction enzyme for SEQ ID NO: 31 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 32 (reverse PCR primer)	64
Restriction enzyme for SEQ ID NO: 32 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 27	1335
Number of amino acid residues in SEQ ID NO: 28	444
Number of different nucleic acid residues between SEQ ID NO: 27 and SEQ ID NO: 29	1
Number of different amino acid residues between SEQ ID NO: 28 and SEQ ID NO: 30	0
Calculated molecular weight of SEQ ID NO: 28 polypeptide (kDa)	49.3
Calculated pI of SEQ ID NO: 28 polypeptide	4.7
Solubility of SEQ ID NO: 30 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one third
Amount of purified polypeptide having SEQ ID NO: 30, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified has the additional amino acid residues from the removed His tag at the N-terminus as described in EXAMPLE 6.	68.1
Amount of purified selmet labeled polypeptide having SEQ ID NO: 30, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified has the additional amino acid residues from the removed His tag at the N-terminus as described in EXAMPLE 6.	2.4
Amount of purified polypeptide having SEQ ID NO: 30 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	123.8
Amount of purified selmet labeled polypeptide having SEQ ID NO: 30 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	6.8

**FIGURE 20-B**

5 **TABLE 3 continued: Properties of UDP-N-acetylmuramate-alanine ligase from *S. aureus***

TABLE 3 -- UDP-N-acetylmuramate-alanine ligase from <i>S. aureus</i> -- SEQ ID NO: 27-SEQ ID NO: 30	
Z-score for the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 30, determined as described in EXAMPLE 9	7.9E-09
Number of matched peptides in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 30, determined as described in EXAMPLE 9	23
Minimum sequence coverage in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 30, determined as described in EXAMPLE 9	42
Calculated molecular weight of SEQ ID NO: 28 polypeptide (Da), determined as described in EXAMPLE 10	52117
Experimental molecular weight of SEQ ID NO: 30 polypeptide (Da), determined as described in EXAMPLE 10	52132
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. The identity of interacting proteins identified by using at least one of the methods described in those examples are: aerobic glycerol-3-phosphate dehydrogenase (gi 13701101), and glutamate racemase (gi 13700950).	
Crystals of a polypeptide having the sequence of SEQ ID NO: 30, prepared and purified as described above and having the residual amino acid residues after removal of the His tag, are obtained using the following conditions: PEG 4000 30%, TRIS 0.1M pH 8.5, sodium acetate 0.2M. The crystals were prepared using the following method: 20°C, sitting drop, 10 mg polypeptide per ml of solution.	

**FIGURE 20-C****TABLE 3A: Truncated Polypeptides of UDP-N-acetylmutamate-alanine ligase from *S. aureus***

UDP-N-acetylmutamate-alanine ligase from <i>S. aureus</i> -- SEQ ID NO: 27-SEQ ID NO: 30							
Start of truncated polypeptide of SEQ ID NO: 30	Y11	F5	I7	T9	Y11		
End of truncated polypeptide of SEQ ID NO: 30	D436	L438	L438	N442	M440		
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approximately two-thirds	Approximately two-thirds	Approximately two-thirds	Approximately two-thirds	Approximately two-thirds		
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	No discernable expression						
Amount of purified truncated polypeptide, prepared and purified as described in the Exemplification (mg/L of culture).	5.0 (1)	4.0 (1)	32.0 (2)	7.0 (1)	25 (2)		
Amount of purified, truncated polypeptide soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	16.0	15.0	32.0	25.0	44		
Z-score for the peptide fingerprint mapping analysis of truncated polypeptide, determined as described in EXAMPLE 9	6.6E-08		6.6E-09	2.2E-07			

**FIGURE 20-D****TABLE 3A continued: Truncated Polypeptides of UDP-N-acetylmuramate-alanine ligase from *S. aureus***

Number of matched peptides in the peptide fingerprint mapping analysis of truncated polypeptide, determined as described in EXAMPLE 9	16	22	24	
Minimum sequence coverage in the peptide fingerprint mapping analysis of truncated polypeptide, determined as described in EXAMPLE 9	36%	55%	44%	
Calculated molecular weight of truncated polypeptide (Da), determined as described in EXAMPLE 10	50090	49393	49062	48768
Experimental molecular weight of truncated polypeptide (Da), determined as described in EXAMPLE 10	50226	49763	49817	50761

(1) The polypeptide so expressed and purified has the additional amino acid residues from the removed His tag at the N-terminus as described in EXAMPLE 6. (2) The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.

UDP-N-acetylmuramate-alanine ligase from <i>S. aureus</i> -- SEQ ID NO: 27-SEQ ID NO: 30			
Start of truncated polypeptide of SEQ ID NO: 30	Y11	Y11	T9
End of truncated polypeptide of SEQ ID NO: 30	L438	N442	M440
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one-third	Approximately two-thirds	Approaching one-third
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	No discernable expression		



FIGURE 20-E

TABLE 3 continued: Truncated Polypeptides of UDP-N-acetylmuramate-alanine ligase from *S. aureus*

UDP-N-acetylmuramate-alanine ligase from <i>S. aureus</i> -- SEQ ID NO: 27-SEQ ID NO: 30					
Start of truncated polypeptide of SEQ ID NO: 30	K3	K3	F5	F5	F5
End of truncated polypeptide of SEQ ID NO: 30	L438	M440	D436	D436	Y434
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one-third	Approaching one-third	Approaching one-third	Approaching one-third	Approaching one-third
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	No discernable expression				

UDP-N-acetylmuramate-alanine ligase from <i>S. aureus</i> -- SEQ ID NO: 27-SEQ ID NO: 30					
Start of truncated polypeptide of SEQ ID NO: 30	F5	F5	T9	T9	I7
End of truncated polypeptide of SEQ ID NO: 30	M440	N442	L438	L438	D436
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one-third	Approaching one-third	Approaching one-third	Approaching one-third	Approaching one-third

UDP-N-acetylmuramate-alanine ligase from <i>S. aureus</i> -- SEQ ID NO: 27-SEQ ID NO: 30					
Start of truncated polypeptide of SEQ ID NO: 30	I7	I7	Y11	Y11	T9
End of truncated polypeptide of SEQ ID NO: 30	M440	N442	Y434	Y434	D436
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one-third	Approaching one-third	No discernable expression	No discernable expression	Approaching one-third
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	No discernable expression	No discernable expression			No discernable expression

## FIGURE 20-F

TABLE 3 continued: Truncated Polypeptides of UDP-N-acetylmuramate-alanine ligase from *S. aureus*

PCR primers and restriction enzymes used to prepare the truncated polypeptides of TABLE 3, and the deleted amino acid residues in them, are set forth in the following tables:

Start of truncated polypeptide	K3	F5	I7	T9	Y11
Residues deleted from N-terminus	MS	MSKE	MSKEFY	MSKEFYIM	MSKEFYIMTH
Nucleic acid sequence of forward PCR primer	SEQ ID NO: 33 G CGCGGGCCCA TATGAAGGAG TTTATATAAT GAC	SEQ ID NO: 34 G CGCGGGCCCA TATGTTTATA TAATGACACA CTATC	SEQ ID NO: 35 G CGCGGGCCCA TATGATAATG ACACACTATC ATTTTG	SEQ ID NO: 36 G CGCGGGCCCA TATGACACACT ATCATTTTGTG G	SEQ ID NO: 37 G CGCGGGCCCA TATGTATCATT TTGTCGGAATT AAAG
Restriction enzyme for forward PCR primer	NdeI	NdeI	NdeI	NdeI	NdeI

End of truncated polypeptide	N442	M440	L438	D436	Y434
Residues deleted from C-terminus	AF	KNAF	GMKNAF	KLGMKNAF	LDKLGKNAF
Nucleic acid sequence of reverse PCR primer	SEQ ID NO: 38 G CGCGGATCCA TTTTTCATGCC TAATTATCTA AATATG	SEQ ID NO: 39 G CGCGGATCCC ATGCCTAATT ATCTAAATATG	SEQ ID NO: 40 G CGCGGATCCT AATTATCTAA ATATGCATTT GTAATTTTG	SEQ ID NO: 41 G CGCGGATCCA TCTAAATATGC ATTTGTAAAT TTTG	SEQ ID NO: 42 G CGCGGATCCA TATGCATTTG TAATTTTGAA TATC
Restriction enzyme for reverse PCR primer	BamHI	BamHI	BamHI	BamHI	BamHI

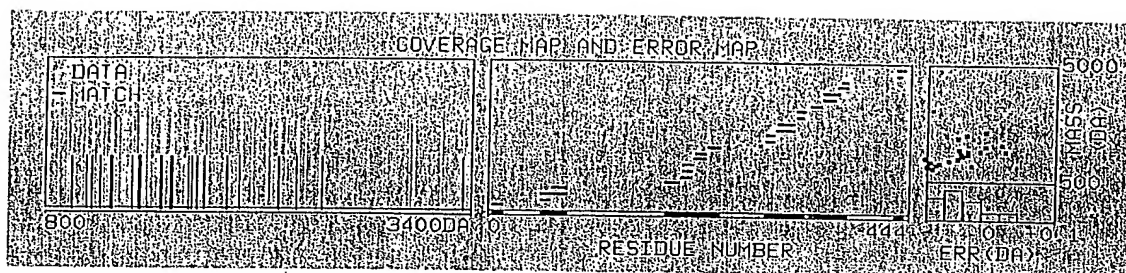
A blank in any of the parts of TABLE 3 indicates that the experiment was not completed.

**FIGURE 21****TABLE 4 Bioinformatic Analyses of UDP-N-acetylmuramate-alanine ligase from *S. aureus***

TABLE 4 -- UDP-N-acetylmuramate-alanine ligase from <i>S. aureus</i> -- SEQ ID NO: 27-SEQ ID NO: 30	
COG Category	Cell envelope biogenesis, outer membrane
COG ID Number	COG0773
Is SEQ ID NO: 28 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 28	None
Source organism for closest PDB protein to SEQ ID NO: 28	N/A
e-value for closest PDB Protein to SEQ ID NO: 28	N/A
% Identity between SEQ ID NO: 28 and the closest protein from PDB	N/A
% Positives between SEQ ID NO: 28 and the closest protein from PDB	N/A
Number of Protein Hits in the VGDB to SEQ ID NO: 28	15
Number of Microorganisms having VGDB Hits to SEQ ID NO: 28	11
Microorganisms having VGDB Hits to SEQ ID NO: 28 <sup>1</sup>	[saur][bsub][efae][spne][hinf][ecoli][rpxx][nmen][paer][bbur][hpyl]
First predicted epitopic region of SEQ ID NO: 28: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 43 :HKEVVAVFQPHT, 1.198,340->351
Second predicted epitopic region of SEQ ID NO: 28: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 44 :KADRVFLCEIFGS, 1.176,368->380,
Third predicted epitopic region of SEQ ID NO: 28: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 45 :DHTVLNALAVIAISYLEKL, 1.168,269->287

- 5                   <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Eschericia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpxx* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

FIGURE 22



Note: click on the ☐ symbol to change column format.

Measured	Avg/	Computed	Error	<input type="checkbox"/> Residues	Missed		
Mass (M)	Mono	Mass	(Da)	Start	To	Cut	Peptide sequence
963.401	M	963.502	-0.101	430	437	0	LQAYLKD
1049.429	M	1049.539	-0.109	294	303	0	EALETFGGVK
1089.533	M	1089.566	-0.033	326	335	0	EISATIETAR
1143.532	M	1143.628	-0.096	57	66	0	ILPFDANNIK
1205.552	M	1205.640	-0.088	294	304	1	EALETFGGVKR
1217.557	M	1217.661	-0.104	326	336	1	EISATIETARK
1339.621	M	1339.695	-0.074	372	382	0	VFLCEIFGSIR
1380.622	M	1380.678	-0.056	206	217	0	GIIAWGDDEHLR
1392.631	M	1392.743	-0.112	430	441	1	LQAYLDKLGHK
1508.723	M	1508.773	-0.050	205	217	1	KGIIAWGDDEHLR
1508.723	M	1508.773	-0.050	206	218	1	GIIAWGDDEHLR
1515.735	M	1515.783	-0.048	342	354	0	EVVAVFQPHFSR
1576.742	M	1576.781	-0.039	219	231	0	IEADVPIYYGFK
1583.731	M	1583.782	-0.051	355	368	0	TQAFLNFAESLSK
1681.844	M	1681.860	-0.016	369	382	1	ADRVFLCEIFGSIR
1704.838	M	1704.876	-0.038	218	231	1	KIEADVPIYYGFK
1737.831	M	1737.805	0.026	232	246	0	DSDDIYAQNIQITDK
1783.819	M	1783.875	-0.055	4	17	0	EFYINTHYHFVGIK
1925.940	M	1925.948	-0.007	355	371	1	TQAFLNFAESLSKADR
1991.925	M	1991.904	0.021	188	204	0	DINDVFDAFQEMAHNVK
2230.011	M	2230.068	-0.057	67	86	0	EDMVVIQGNAPASSHEEIVR
2339.088	M	2339.129	-0.041	306	325	0	FNETTIANQVIVDDYAHHPR
2495.221	M	2495.229	-0.008	305	325	1	RFNETTIANQVIVDDYAHHPR
3355.585	M	3355.686	-0.100	57	86	1	ILPFDANNIKEDMVVIQGNAPASSHEEIVR

FIGURE 23

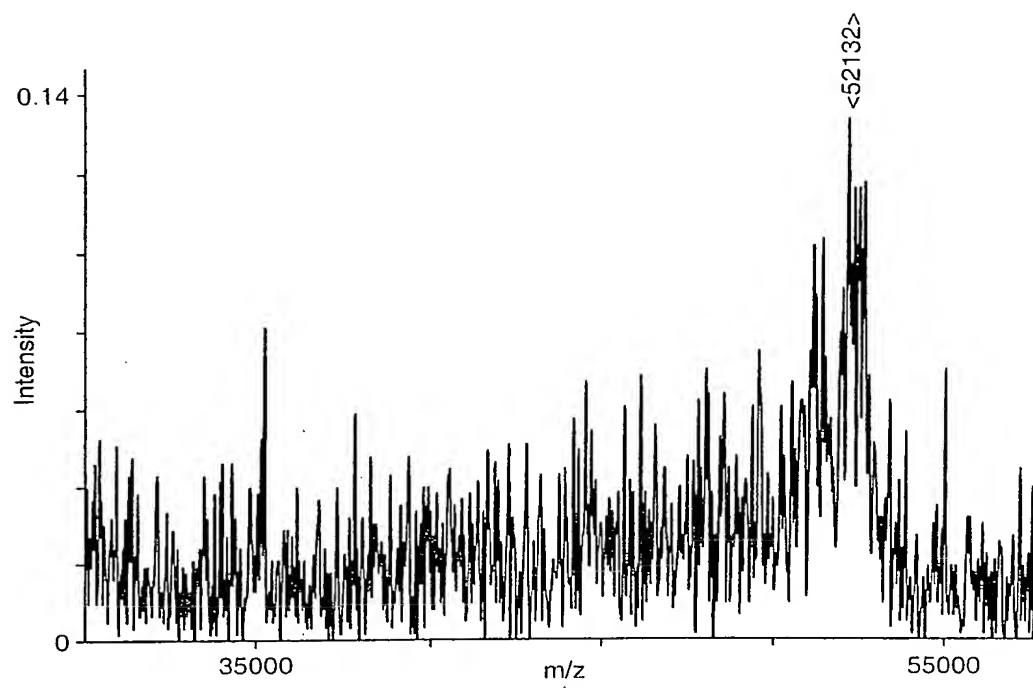


FIGURE 24

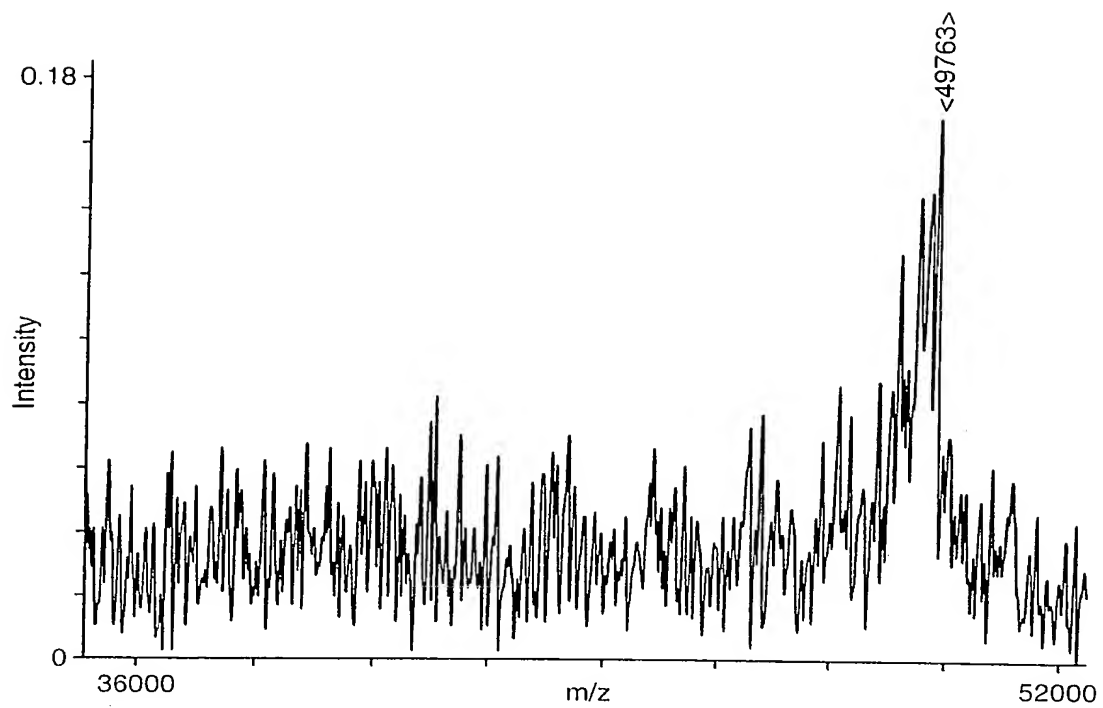


FIGURE 25

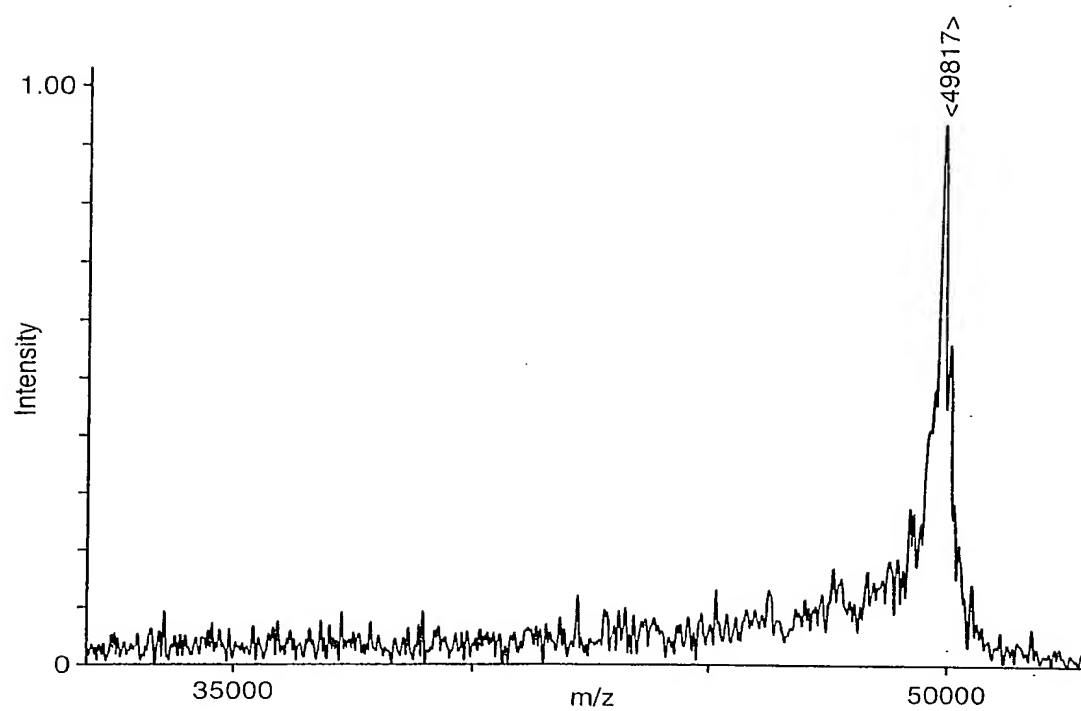


FIGURE 26



Note: click on the ☐ symbol to change column format.

Measured Mass (M)	Avg/ Mono	Computed Mass	Error (Da)	<input type="checkbox"/> Residues Start	<input type="checkbox"/> Residues To	<input type="checkbox"/> Residues Cut	Hissed Peptide sequence
1049.501	M	1049.539	-0.037	294	303	0	EALETFGGVK
1089.556	M	1089.566	-0.010	326	335	0	EISATITETAR
1143.605	M	1143.628	-0.023	57	66	0	ILPFDANNIK
1205.579	M	1205.640	-0.061	294	304	1	EALETFGGVKR
1217.714	M	1217.661	0.053	326	336	1	EISATITETARK
1339.675	M	1339.695	-0.020	372	382	0	VFLCEIFGSIR
1380.677	M	1380.678	-0.001	206	217	0	GIIAWGDDEHLR
1515.791	M	1515.783	0.008	342	354	0	EVVAVFQPHFTR
1583.819	M	1583.782	0.036	355	368	0	TQAFLEFAESLSK
1681.936	M	1681.860	0.075	369	382	1	ADRVFLCEIFGSIR
1704.932	M	1704.876	0.056	218	231	1	KIEADVPIYYGFK
1737.862	M	1737.805	0.057	232	246	0	DSDDIYAQHIQITDK
1783.819	M	1783.875	-0.056	4	17	0	EPYINTHYHFGIK
1925.975	M	1925.948	0.027	355	371	1	TQAFLEFAESLSKADR
1991.955	M	1991.904	0.051	188	204	0	DINDVFDAFQEMAHNVK
2041.168	M	2041.053	0.115	338	354	1	YPHKEVVAVFQPHFTR
2120.158	M	2119.999	0.159	188	205	1	DINDVFDAFQEMAHNVK
2230.115	M	2230.068	0.047	67	86	0	EDMVVIQGNFASSHEEIVR
2339.196	M	2339.129	0.068	306	325	0	FNETTIANQVIVDDYAHHP
2495.333	M	2495.229	0.104	305	325	1	RNETTIANQVIVDDYAHHP
2976.462	M	2976.378	0.084	164	187	0	HFLSYKPDYAINTNIDFDHPDYFK
3032.385	M	3032.319	0.066	136	162	0	TSFLIGDGTGMGLPESDYFAFEACEYR



FIGURE 27

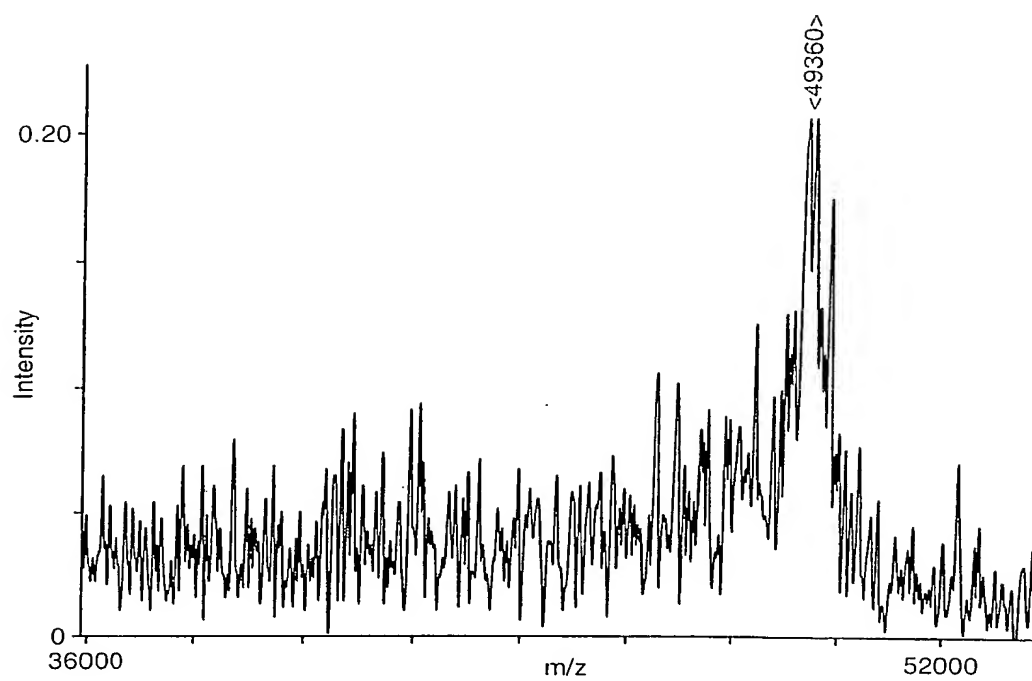
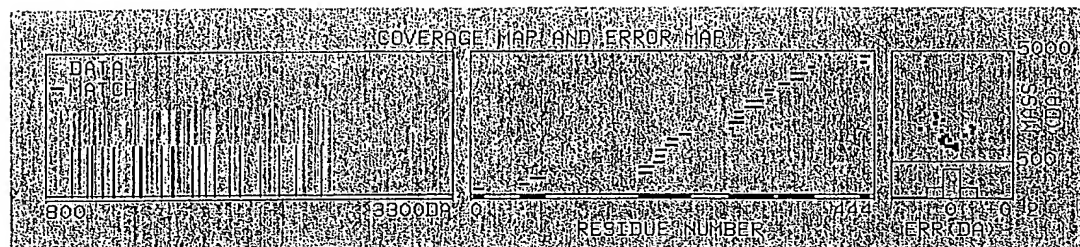


FIGURE 28



Note: click on the ☐ symbol to change column format.

Measured Mass (M)	Avg/ Mono	Computed Mass	Error <input type="checkbox"/> (Da)	<input type="checkbox"/> Residues Start	Missed To	Cut	Peptide sequence
801.295	M	801.459	-0.165	287	293	0	LDVTNIK
963.516	M	963.502	0.014	430	437	0	LQNAYLDK
1049.549	M	1049.539	0.010	294	303	0	EALETFGGVK
1089.555	M	1089.566	-0.010	326	335	0	EISATLETAR
1143.630	M	1143.628	0.002	57	66	0	ILPFDANNIK
1205.656	M	1205.640	0.016	294	304	1	EALETFGGVK
1217.636	M	1217.661	-0.025	326	336	1	EISATLETARK
1339.676	M	1339.695	-0.019	372	382	0	VFLCEIFGSIR
1380.649	M	1380.678	-0.029	206	217	0	GIIAWGDDEHLR
1392.740	M	1392.743	-0.003	430	441	1	LQNAYLCLKGNK
1508.750	M	1508.773	-0.022	205	217	1	KGIIAWGDDEHLR
1508.750	M	1508.773	-0.022	206	218	1	GIIAWGDDEHLR
1515.762	M	1515.783	-0.021	342	354	0	EVVAVFQPHFSR
1576.771	M	1576.781	-0.009	219	231	0	TEADVPIYYGFK
1583.820	M	1583.782	0.037	355	368	0	TQAFLEFAESLSK
1704.931	M	1704.876	0.056	218	231	1	KTEADVPIYYGFK
1737.863	M	1737.805	0.058	232	246	0	DSDDIYAQNIQITDK
1783.852	M	1783.875	-0.023	4	17	0	EFYIMTHYHFVGK
1832.904	M	1832.987	-0.083	287	303	1	LDVTNIKEALETFGGVK
1926.008	M	1925.948	0.060	355	371	1	TQAFLEFAESLSKADR
1991.957	M	1991.904	0.053	188	204	0	DINDVFDQFQEMAHNVK
2119.955	M	2119.999	-0.043	188	205	1	DINDVFDQFQEMAHNVK
2230.047	M	2230.068	-0.021	67	86	0	EDMVVIQGNFASSHIEIVR
2339.089	M	2339.129	-0.039	306	325	0	FNETTIANQVIYDDYAHHP
2495.184	M	2495.229	-0.046	305	325	1	RNETTIANQVIYDDYAHHP

FIGURE 29

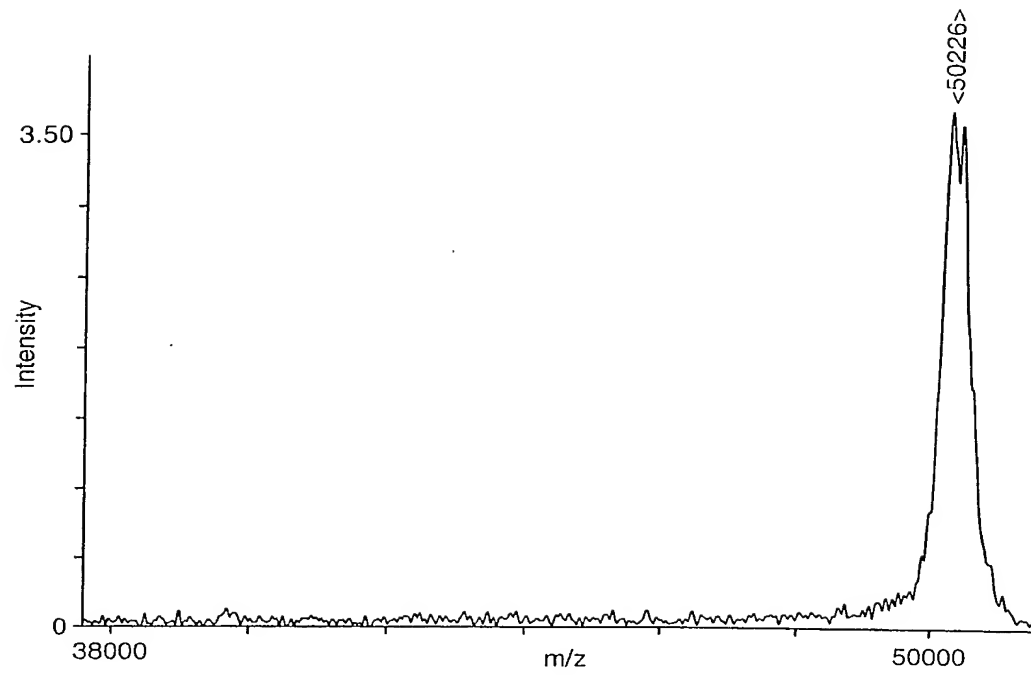
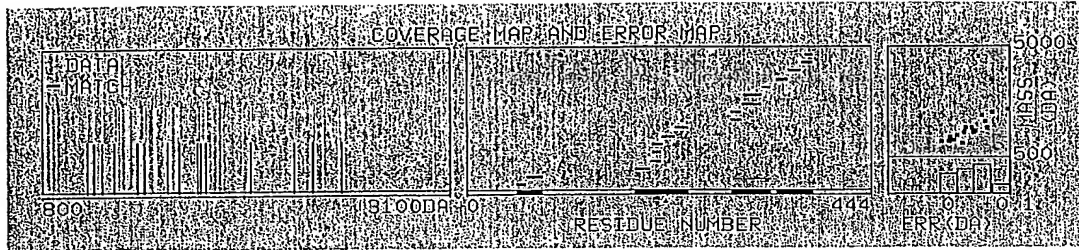


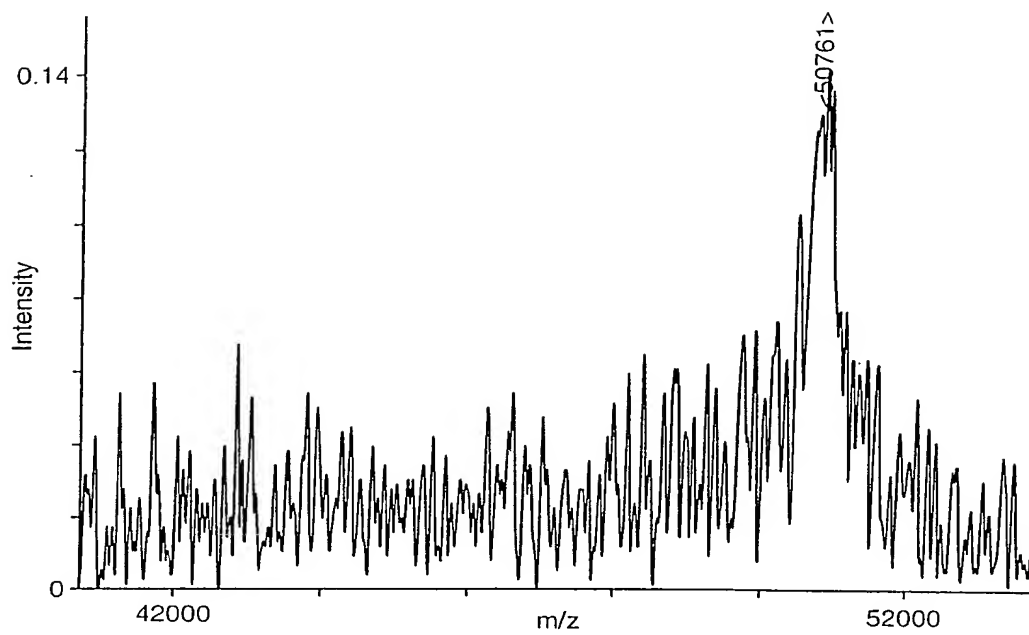
FIGURE 30



Note: click on the ☐ symbol to change column format.

Measured Mass (M)	Avg/ Mono	Computed Mass	Error <input type="checkbox"/> (Da)	<input type="checkbox"/> Residues Start	Missed To	Cut	Peptide sequence
1049.570	M	1049.539	0.032	294	303	0	EALETFGGVK
1089.553	M	1089.566	-0.013	326	335	0	EISATIETAR
1143.630	M	1143.628	0.001	57	66	0	ILPFDANNIK
1205.652	M	1205.640	0.012	294	304	1	EALETFGGVKR
1339.702	M	1339.695	0.007	372	382	0	VFLCEIFGSIR
1380.700	M	1380.678	0.022	206	217	0	GIIAWGDDEHLR
1508.832	M	1508.773	0.059	206	218	1	GIIAWGDDEHLR
1508.832	M	1508.773	0.059	205	217	1	KGIIAWGDDEHLR
1515.818	M	1515.783	0.036	342	354	0	EVVAVFQPHFSR
1583.844	M	1583.782	0.061	355	368	0	TQAFLEFAESLSK
1681.902	M	1681.860	0.041	369	382	1	ADRVFLCEIFGSIR
1704.930	M	1704.876	0.054	218	231	1	KIEADVPIYYGFK
1737.893	M	1737.805	0.089	232	246	0	DSDDIYAQNIQITDK
1991.988	M	1991.904	0.084	188	204	0	DINDVFDAFQEMAHNVK
2230.146	M	2230.068	0.078	67	86	0	EDHVVIQGNFASSHEEIVR
2339.229	M	2339.129	0.101	306	325	0	FNETTIANQVIVDDYAHHPR
2495.365	M	2495.229	0.135	305	325	1	RNETTIANQVIVDDYAHHPR

FIGURE 31



**FIGURE 32**

SEQ ID NO: 46

GTGATAAATAAAGACATCTATCAAGCTTTACAACAACCTTATCCCAAATG  
5 AAAAAATTAAAGTTGATGAACCTTTAAAACGATACACTTATACTAAAACAGGT  
GGTAATGCCGACTTTTACATTACCCCTACTAAAAATGAAGAAGTACAAGCAGTT  
GTTAAATATGCCTATCAAAATGAGATTCCTGTTACATATTTAGGAAATGGCTCA  
AATATTATTATCCGTGAAGGTGGTATTCGCGGTATTGTAATTAGTTTATTATCAC  
TAGATCATATCGAAGTATCTGATGATGCGATAATAGCCGGTAGCGGCGCTGCA  
10 ATTATTGATGTCTCACGTGTTGCTCTTGATTACGCACTTACTGGCCTTGAATTTG  
CATGTGGTATTCCAGGTTCAATTGGTGGTGCAGTGTATATGAATGCTGGCGCTT  
ATGGTGGCGAAGTTAAAGATTGTATAGACTATGCGCTTTGCGTAAACGAACAA  
GGCTCGTTAATTAAACTTACAACAAAAGAATTAGAGTTAGATTATCGTAATAGC  
ATTATTCAAAAAGAACAACCTTAGTTGTATTAGAAGCTGCATTTACTTTAGCTCCT  
15 GGTAATGACTGAAATACAAGCTAAAATGGATGATTTAACAGAACGTAGAGA  
ATCTAAACAACCTTTAGAGTATCCTTCATGTGGTAGTGTATTCCAAAGACCGCC  
TGGTCATTTTGCAGGTAAATTGATACAAGATTCTAATTTGCAAGGTCACCGTAT  
TGGCGGCGTTGAAGTTTCAACCAAACACGCTGGTTTTATGGTAAATGTAGACAA  
TGGAAGTCTACAGATTATGAAAACCTTATTCATTATGTACAAAAGACCGTCAA  
20 AGAAAAATTTGGCATTGAATTAAATCGTGAAGTTCGCATTATTGGTGAACATCC  
AAAGGAATCGTAA

**FIGURE 33**

SEQ ID NO: 47

VINKDIYQALQQLIPNEKIKVDEPLKRYTYTKTGGNADFYITPTKNEEVQAV  
5 VKYAYQNEIPVTYLGNGSNIIREGGIRGIVISLLSLDHIEVSDDAIAGSGAAIDVSR  
VALDYALTGLEFACGIPGSIGGAVYMNAGAYGGEVKDCIDYALCVNEQGSLIKLT  
KELELDYRNSIIQKEHLVVLEAAFTLAPGKMTEIQAKMDDLTERRESKQPLEYPSC  
GSVFQRPPGHFAGKLIQDSNLQGHRIGGVEVSTKHAGFMVNVNDNGTATDYENLIH  
YVQKTVKEKFGIELNREVRIIGEHPKES

**FIGURE 34**

SEQ ID NO: 48

GTGATAAATAAAGACATCTATCAAGCTTTACAACAACCTTATCCCAAATG  
5 AAAAAATTAAAGTTGATGAACCTTTAAAACGATACACTTATACTAAAACAGGT  
GGTAATGCCGACTTTTACATTACCCCTACTAAAAATGAAGAAGTACAAGCAGTT  
GTTAAATATGCCTATCGAAATGAGATTCCTGTTACATATTTAGGAAATGGCTCA  
AATATTATTATCCGTGAAGGTGGTATTCGCGGTATTGTAATTAGTTTATTACCAC  
TAGATCATATCGAAGTATCTGATGATGCGATAATAGCCGGTAGCGGCGCTGCA  
10 ATTATTGATGTCTCACGTGTTGCTCGTGATTACGCACTTACTGGCCTTGAATTTG  
CATGTGGTATTCCAGGTTCAATTGGTGGTGCAGTGTATATGAATGCTGGCGCTT  
ATGGTGGCGAAGTTAAAGATTGTATAGACTATGCGCTTTGCGTAAACGAACAA  
GGCTCGTTAATTAAACTTACAACAAAAGAATTAGAGTTAGATTATCGTAATAGC  
ATTATTCAAAAAGAACACTTAGTTGTATTAGAAGCTGCATTTACTTTAGCTCCT  
15 GGTAATGACTGAAATACAAGCTAAAATGGATGATTTAACAGAACGTAGAGA  
ATCTAAACAACCTTTAGAGTATCCTTCATGTGGTAGTGTATTCCAAAGACCGCC  
TGGTCATTTTGCAGGTAAATTGATACAAGATTCTAATTTGCAAGGTCACCGTAT  
TGGCGGCGTTGAAGTTTCAACCAAACACGCTGGTTTTATGGTAAATGTAGACAA  
TGGAAGTCTACAGATTATGAAAACCTTATTCATTATGTACAAAAGACCGTCAA  
20 AGAAAAATTTGGCATTGAATTAAATCGTGAAGTTCGCATTATTGGTGAACATCC  
AAAGGAATCGTAA



**FIGURE 35**

SEQ ID NO: 49

VINKDIYQALQQLIPNEKIKVDEPLKRYTYTKTGGNADFYITPTKNEEVQAV  
5 VKYAYRNEIPVTYLGNGSNIIREGGIRGIVISLLPLDHIEVSDDAILAGSGAAIIDVSR  
VARDYALTGLEFACGIPGSIGGAVYMNAGAYGGEVKDCIDYALCVNEQGSLIKLT  
TKELELDYRNSIIQKEHLVVLEAAFTLAPGKMTEIQAKMDDLTERRESKQPLEYPSC  
GSVFQRPPGHFAGKLIQDSNLQGHRIGGVEVSTKHAGFMVNVDNGTATDYENLIH  
YVQKTVKEKFGIELNREVRIIGEHPKES

**FIGURE 36**

SEQ ID NO: 50

Forward PCR Primer

5 GCGGCGGCCCATATGGATAACTACACCTATAGC

SEQ ID NO: 51

10

Reverse PCR Primer

GCGCGGATCCTTAGAGTTCAAACAATTCTACGCTTTC

**FIGURE 37****TABLE 5 Properties of UDP-N-acetylenolpyruvylglucosamine reductase from *S. aureus***

TABLE 5 -- UDP-N-acetylenolpyruvylglucosamine reductase from <i>S. aureus</i> -- SEQ ID NO: 46-SEQ ID NO: 49	
Melting temperature (°C) of SEQ ID NO: 50 (forward PCR primer)	58
Restriction enzyme for SEQ ID NO: 50 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 51 (reverse PCR primer)	66
Restriction enzyme for SEQ ID NO: 51 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 46	924
Number of amino acid residues in SEQ ID NO: 47	307
Number of different nucleic acid residues between SEQ ID NO: 46 and SEQ ID NO: 48	3
Number of different amino acid residues between SEQ ID NO: 47 and SEQ ID NO: 49	3
Calculated molecular weight of SEQ ID NO: 47 polypeptide (kDa)	34.1
Calculated pI of SEQ ID NO: 47 polypeptide	5
Solubility of SEQ ID NO: 49 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one third
Amount of purified polypeptide having SEQ ID NO: 49, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified has the additional amino acid residues from the removed His tag at the N-terminus as described in EXAMPLE 6.	24.7
Amount of purified polypeptide having SEQ ID NO: 49 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	16.5

**FIGURE 37-B**

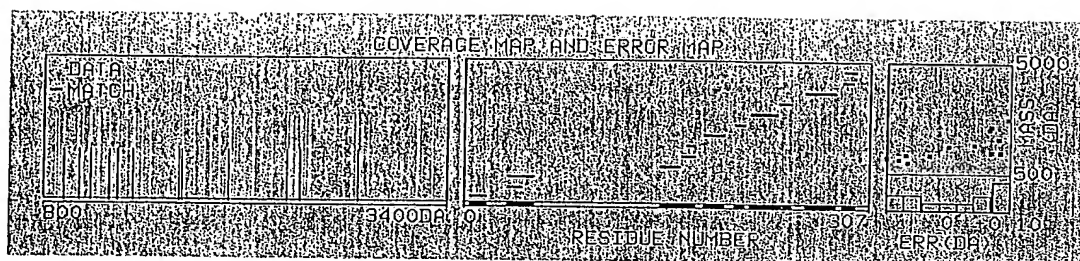
TABLE 5 -- UDP-N-acetylenolpyruvylglucosamine reductase from <i>S. aureus</i> -- SEQ ID NO: 46-SEQ ID NO: 49	
Z-score for the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 49, determined as described in EXAMPLE 9	8.7E-06
Number of matched peptides in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 49, determined as described in EXAMPLE 9	13
Minimum sequence coverage in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 49, determined as described in EXAMPLE 9	51
Calculated molecular weight of SEQ ID NO: 47 polypeptide (Da), determined as described in EXAMPLE 10	35753
Experimental molecular weight of SEQ ID NO: 49 polypeptide (Da), determined as described in EXAMPLE 10	36075
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. No interacting proteins were observed by using at least one of the methods described in those examples.	
Crystals of a polypeptide having the sequence of SEQ ID NO: 49, prepared and purified as described above and having the residual amino acid residues after removal of the His tag, are obtained using the following conditions: 24% PEG4000, HEPES pH 7.5, 0.2M ammonium sulfate. The crystals were prepared using the following method: 20°C, sitting drop, 15 mg polypeptide per ml of solution.	

**FIGURE 38****TABLE 6 Bioinformatic Analyses of UDP-N-acetylenolpyruvylglucosamine reductase from *S. aureus***

TABLE 6 -- UDP-N-acetylenolpyruvylglucosamine reductase from <i>S. aureus</i> -- SEQ ID NO: 46-SEQ ID NO: 49	
COG Category	Cell envelope biogenesis, outer membrane
COG ID Number	COG0812
Is SEQ ID NO: 47 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 47	UDP-N-Acetylenolpyruvylglucosamine Reductase (1hsk)
Source organism for closest PDB protein to SEQ ID NO: 47	<i>Staphylococcus aureus</i>
e-value for closest PDB Protein to SEQ ID NO: 47	E-176
% Identity between SEQ ID NO: 47 and the closest protein from PDB	99
% Positives between SEQ ID NO: 47 and the closest protein from PDB	99
Number of Protein Hits in the VGDB to SEQ ID NO: 47	8
Number of Microorganisms having VGDB Hits to SEQ ID NO: 47	8
Microorganisms having VGDB Hits to SEQ ID NO: 47 <sup>1</sup>	[spne][saur][bsub][efae] [bbur][rpxx][ctra][hpyl]
First predicted epitopic region of SEQ ID NO: 47: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 52 :VQAVVKYA, 1.188,49->56
Second predicted epitopic region of SEQ ID NO: 47: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 53 :VKDCIDYALCVNEQ, 1.188,147->160
Third predicted epitopic region of SEQ ID NO: 47: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 54 :RGIVISLLSLDHIEVSD-DAIAGSGAAIDVSRVALDYALTGLE-FACGIPGS, 1.187,80->131

- 5                   <sup>1</sup>Organisms are abbreviated as follows: ecoli = *Eschericia coli*; hpyl = *Helicobacter pylori*; paer = *Pseudomonas aeruginosa*; ctra = *Chlaydia trachomatis*; hinf = *Haemophilus influenzae*; nmen = *Neisseria meningitidis*; rpxx = *Rickettsia prowazekii*; bbur = *Borrelia burgdorferi*; bsub = *Bacillus subtilis*; staph = *Staphylococcus aureus*; spne = *Streptococcus pneumoniae*; mgen = *Mycoplasma genitalium*; efae = *Enterococcus faecalis*.

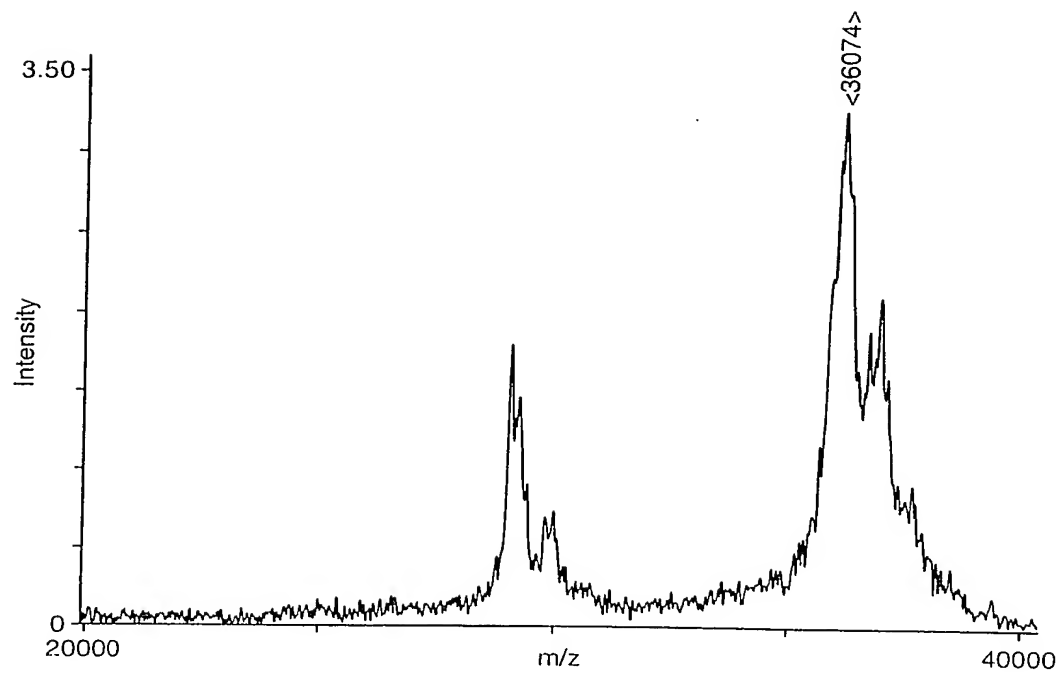
FIGURE 39



Note: click on the ☐ symbol to change column format.

Measured Mass (k)	Avg/ Mono	Computed Mass	Error (Da)	<input type="checkbox"/> Residues Start	<input type="checkbox"/> Missed To	Cut	Peptide sequence
936.392	H	936.455	-0.063	170	176	0	ELELDYR
1034.406	M	1034.481	-0.075	206	213	1	HDDLERR
1104.497	M	1104.592	-0.095	287	295	1	EKFGIELNR
1231.640	M	1231.667	-0.027	289	298	1	FGIELNREVR
1279.597	M	1279.663	-0.066	239	249	0	LIQDSNLQGH
1379.809	M	1379.729	0.080	166	176	1	LTKELELDYR
1383.735	M	1383.666	0.068	33	45	0	TGGNADFYITPTK
1671.885	M	1671.882	0.002	5	18	0	DIYQALQLIPNEK
1693.979	M	1693.940	0.040	183	198	0	EHLVVLEAFTLAPGK
1997.000	M	1996.922	0.077	149	165	0	DCIDYALCVNEQGSLLK
2380.255	H	2380.190	0.065	33	54	1	TGGNADFYITPTKNEEVQAVVK
2458.252	M	2458.184	0.068	217	238	0	QPLEYPSGCVFQRPFGHFAGK
2835.419	M	2835.328	0.092	259	283	0	HAGFMVNDNGTATDYENLIHYVQK

FIGURE 40



**FIGURE 41**

SEQ ID NO: 55

ATGACAAGAAAAGGATATGGGGAATCGACAGGTAAGATTATTTTAATAG  
5 GAGAACATGCTGTTACATTTGGAGAGCCTGCTATTGCAGTACCGTTTAACGCAG  
GTAAAATCAAAGTTTTAATAGAAGCCTTAGAGAGCGGGAACCTATTCGTCTATTA  
AAAGCGATGTTTACGATGGTATGTTATATGATGCGCCTGACCATCTTAAGTCTT  
TGGTGAACCGTTTTGTAGAATTAAATAATATTACAGAGCCGCTAGCAGTAACGA  
TCCAAACGAATTTACCACCATCACGTGGATTAGGATCGAGTGCAGCTGTCGCGG  
10 TTGCTTTTGTTCGTGCAAGTTATGATTTTTTTAGGGAAATCATTAACGAAAGAAG  
AACTCATTGAAAAGGCTAATTGGGCAGAGCAAATTGCACATGGTAAACCAAGT  
GGTATTGATACGCAAACGATTGTATCAGGCAAACCAAGTTTGGTTCCAAAAAGG  
TCATGCTGAAACGTTGAAAACGTTAAGTTTAGACGGCTATATGGTTGTTATAGA  
TACTGGTGTGAAAGGTTCAACAAGACAAGCAGTAGAAGATGTTCATAAACTTT  
15 GTGAGGACCCTCAGTACATGTCACATGTAAAACATATCGGTAAGTTAGTTTTAC  
GTGCGAGTGATGTGATTGAACATCATAACTTTGAAGCCTTAGCGGATATTTTAA  
ATGAATGTCATGCGGATTTAAAGGCGTTGACAGTTAGTCATGATAAAATAGAA  
CAATTAATGAAAATTGGTAAAGAAAATGGTGCGATTGCTGGAAAACCTTACTGG  
CGCTGGTCGTGGTGGAAGTATGTTATTGCTTGCCAAAGATTTACCAACAGCGAA  
20 AAATATTGTAAAAGCTGTAGAAAAAGCTGGTGCAGCACATACTTGGATTGAGA  
ATTTAGGAGGTAA



**FIGURE 42**

SEQ ID NO: 56

MTRKGYGESTGKIILIGEHAVTFGEPAIAVPFNAGKIKVLIEALESIGNYSSIKS  
5 D V Y D G M L Y D A P D H L K S L V N R F V E L N N I T E P L A V T I Q T N L P P S R G L G S S A A V A V A F V  
R A S Y D F L G K S L T K E E L I E K A N W A E Q I A H G K P S G I D T Q T I V S G K P V W F Q K G H A E T L K  
T L S L D G Y M V V I D T G V K G S T R Q A V E D V H K L C E D P Q Y M S H V K H I G K L V L R A S D V I E H  
H N F E A L A D I F N E C H A D L K A L T V S H D K I E Q L M K I G K E N G A I A G K L T G A G R G G S M L L L  
A K D L P T A K N I V K A V E K A G A A H T W I E N L G G

**FIGURE 43**

SEQ ID NO: 57

ATGACAAGAAAAGGATATGGGGAATCGACAGGTAAGATTATTTTAATAG  
5 GAGAACATGCTGTTACATTTGGAGAGCCTGCTATTGCAGTACCGTTTAACGCAG  
GTAAAATCAAAGTTTTAATAGAAGCCTTAGAGAGCGGGAAGTATTCGTCTATTA  
AAAGCGATGTTTACGATGGTATGTTATATGATGCGCCTGACCATCTTAAGTCTT  
TGGTGAACCGTTTTGTAGAATTAAATAATATTACAGAGCCGCTAGCAGTAACGA  
TCCAAACGAATTTACCACCATCACGTGGATTAGGATCGAGTGCAGCTGTCGCGG  
10 TTGCTTTTGTTCGTGCAAGTTATGATTTTTTTAGGGAAATCATTAACGAAAGAAG  
AACTCATTGAAAAGGCTAATTGGGCAGAGCAAATTGCACATGGTAAACCAAGT  
GGTATTGATACGCAAACGATTGTATCAGGCAAACCAGTTTGGTTCCAAAAAGG  
TCATGCTGAAACGTTGAAAACGTTAAGTTTAGACGGCTATATGGTTGTTATAGA  
TACTGGTGTGAAAGGGTCAACAAGACAAGCAGTAGAAGATGTTCATAAACTTT  
15 GTGAGGACCCTCAGTACATGTCACATGTAAAACATATCGGTAAGTTAGTTTTAC  
GTGCGAGTGATGTGATTGAACATCATAACTTTGAAGCCTTAGCGGATATTTTAA  
ATGAATGTCATGCGGATTTAAAGGCGTTGACAGTTAGTCATGATAAAATAGAA  
CAATTAATGAAAATTGGTAAAGAAAATGGTGCGATTGCTGGAAAACCTTACTGG  
CGCTGGTTCGTGGTGGGAAGTATGTTATTGCTTGCCAAAGATTTACCAACAGCGAA  
20 AAATATTGTAAAAGCTGTAGAAAAAGCTGGTGCAGCACATACTTGGATTGAGA  
ATTTAGGAGGTAA

**FIGURE 44**

SEQ ID NO: 58

MTRKGYGESTGKIILIGEHAVTFGEPAIAVPFNAGKIKVLIEALESGNYSSIKS  
5 D VYDGM LYDAPDHLKSLVNRFVELNNITEPLAVTIQTNLPPSRGLGSSAAVAVAFV  
RASYDFLGKSLTKEELIEKANWAEQIAHGKPSGIDTQTIVSGKPVWFQKGHAETLK  
TSLDGYMVVIDTGVKGSTRQAVEDVHKLCEDPQYMSHVKHIGKLVLRASDVIEH  
HNFEALADIFNECHADLKALTVSHDKIEQLMKIGKENGAIA GKLTGAGRGGSM LLL  
AKDLPTAKNIVKAVEKAGAAHTWIENLGG

10

**FIGURE 45**

SEQ ID NO: 59

Forward PCR Primer

5 GCGGCGGCCCATATGACAAGAAAAGGATATGGG

SEQ ID NO: 60

10

Reverse PCR Primer

GCGCGGATCCCGGCTCTGTAATATTATTTAATTC

**FIGURE 46****TABLE 7 Properties of mevalonate kinase from *S. aureus***

TABLE 7 -- mevalonate kinase from <i>S. aureus</i> -- SEQ ID NO: 55-SEQ ID NO: 58	
Melting temperature (°C) of SEQ ID NO: 59 (forward PCR primer)	58
Restriction enzyme for SEQ ID NO: 59 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 60 (reverse PCR primer)	62
Restriction enzyme for SEQ ID NO: 60 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 55	921
Number of amino acid residues in SEQ ID NO: 56	306
Number of different nucleic acid residues between SEQ ID NO: 55 and SEQ ID NO: 57	1
Number of different amino acid residues between SEQ ID NO: 56 and SEQ ID NO: 58	0
Calculated molecular weight of SEQ ID NO: 56 polypeptide (kDa)	32.9
Calculated pI of SEQ ID NO: 56 polypeptide	6.5
Solubility of SEQ ID NO: 58 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching 100%
Amount of purified polypeptide having SEQ ID NO: 58, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified has the additional amino acid residues from the removed His tag at the N-terminus as described in EXAMPLE 6.	88.0
Amount of purified selmet labeled polypeptide having SEQ ID NO: 58, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	17.4
Amount of purified <sup>15</sup> N labeled polypeptide having SEQ ID NO: 58, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	32.6
Amount of purified polypeptide having SEQ ID NO: 58 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	110.0
Amount of purified selmet labeled polypeptide having SEQ ID NO: 58 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	34.8
Amount of purified <sup>15</sup> N labeled polypeptide having SEQ ID NO: 58 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	36.2

**FIGURE 46-B**

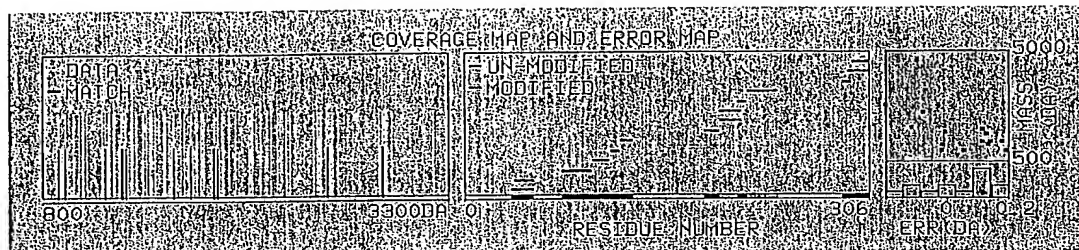
TABLE 7 -- mevalonate kinase from <i>S. aureus</i> -- SEQ ID NO: 55-SEQ ID NO: 58	
Z-score for the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 58, determined as described in EXAMPLE 9	3.7E-04
Number of matched peptides in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 58, determined as described in EXAMPLE 9	12
Minimum sequence coverage in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 58, determined as described in EXAMPLE 9	43
Calculated molecular weight of SEQ ID NO: 56 polypeptide (Da), determined as described in EXAMPLE 10	33480
Experimental molecular weight of SEQ ID NO: 58 polypeptide (Da), determined as described in EXAMPLE 10	33490
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. The identity of an interacting protein identified by using at least one of the methods described in those examples is: ~70 kDa unidentified protein	
Crystals of a polypeptide having the sequence of SEQ ID NO: 58, prepared and purified as described above and having the residual amino acid residues after removal of the His tag, are obtained using the following conditions: 2M Ammonium Sulfate. The crystals were prepared using the following method: 20°C, sitting drop, 10 mg polypeptide per ml of solution.	
Crystals of a selenomethionine-substituted polypeptide having the sequence of SEQ ID NO: 58, prepared and purified as described above and having the residual amino acid residues after removal of the His tag, are obtained using the following conditions: 3M ammonium sulfate, sodium citrate pH 5.5. In addition, crystals of the same polypeptide may be prepared under the following conditions: ammonium sulfate 2M, 5% MPD. Further, crystals of the same polypeptide may be prepared under the following conditions: 1.4M sodium citrate, sodium acetate pH 4.5. The crystals were prepared using the following method: 20°C, sitting drop, 10 mg polypeptide per ml of solution.	

**FIGURE 47****TABLE 8 Bioinformatic Analyses of mevalonate kinase from *S. aureus***

TABLE 8 -- mevalonate kinase from <i>S. aureus</i> -- SEQ ID NO: 55-SEQ ID NO: 58	
COG Category	Lipid metabolism
COG ID Number	COG1577
Is SEQ ID NO: 56 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 56	None
Source organism for closest PDB protein to SEQ ID NO: 56	N/A
e-value for closest PDB Protein to SEQ ID NO: 56	N/A
% Identity between SEQ ID NO: 56 and the closest protein from PDB	N/A
% Positives between SEQ ID NO: 56 and the closest protein from PDB	N/A
Number of Protein Hits in the VGDB to SEQ ID NO: 56	4
Number of Microorganisms having VGDB Hits to SEQ ID NO: 56	4
Microorganisms having VGDB Hits to SEQ ID NO: 56 <sup>1</sup>	[saur][efae][spne][bbur]
First predicted epitopic region of SEQ ID NO: 56: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 61 :SSAAVAVAFVR-ASYDFLGKS, 1.205, 101->120,
Second predicted epitopic region of SEQ ID NO: 56: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 62 :TLKTLSLDGYM-VVIDT, 1.136, 164->179,
Third predicted epitopic region of SEQ ID NO: 56: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 63 :YMSHVKHIGKL-VLRASDVIEH, 1.135, 201->221

<sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Eschericia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpxx* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

FIGURE 48

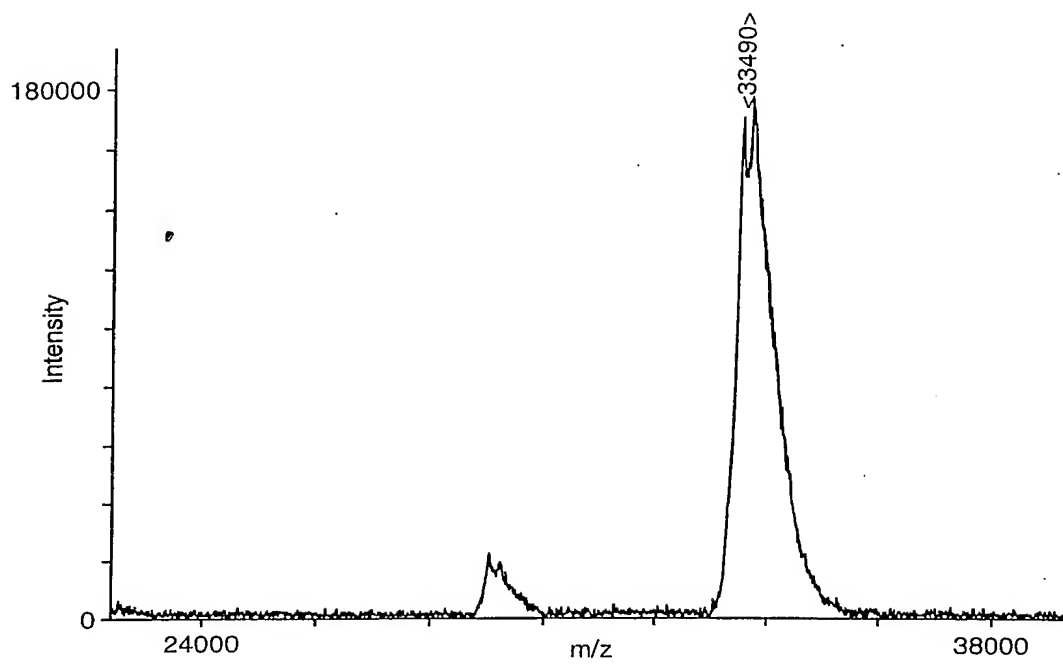


Note: click on the ☐ symbol to change column format.

Measured Mass (M)	Avg/ Mono	Computed Mass	Error (Da)	<input type="checkbox"/> Residues Start	<input type="checkbox"/> Missed To	Cut	Peptide sequence
899.587	M	899.544	0.043	286	293	1	NIVKAVEK
899.587	M	899.438	0.148	112	119	0	ASYDFLGK
934.595	M	934.607	-0.012	207	214	1	HIGKLVLR
1188.763	M	1188.659	0.104	120	129	1	SLTKEELIEK
1295.715	M	1295.625	0.090	294	306	0	AGAHTWIENLGG
1303.832	M	1303.724	0.108	98	111	0	GLGSSAAVAVAFVR
1325.807	M	1325.668	0.139	183	194	1	GSTRQAVEDVHK
1621.956	M	1621.855	0.101	39	53	0	VLIEALESQNYSSIK
1722.866	M	1722.868	-0.001	290	306	1	AVEKAGAHTWIENLGG
1863.123	M	1863.034	0.089	37	53	1	IKVLIEALESQNYSSIK
1883.814	M	1883.901	-0.087	195	210	1	LCEDPQYMSHVKKHIGK (1)+c40N;
1883.814	M	1883.901	-0.087	195	210	1	LCEDPQYMSHVKKHIGK
2565.323	M	2565.379	-0.056	75	97	0	FVELNNITEPLAVTIQTNLPPSR
2894.176	M	2894.328	-0.153	215	239	0	ASDVIEHHNFEALADIFNECHADLK (1)+C2H3C4N6C;



FIGURE 49



**FIGURE 50**

SEQ ID NO: 64

ATGAGTCTGAATTTCTTGATTTTGAACAGCCGATTGCAGAGCTGGAAG  
5 CGAAAATCGATTCTCTGACTGCGGTTAGCCGTCAGGATGAGAAACTGGATATTA  
ACATCGATGAAGAAGTGCATCGTCTGCGTGAAAAAAGCGTAGAACTGACACGT  
AAAATCTTCGCCGATCTCGGTGCATGGCAGATTGCGCAACTGGCACGCCATCCA  
CAGCGTCCTTATACCCTGGATTACGTTTCGCCTGGCATTGATGAATTTGACGAA  
CTGGCTGGCGACCGCGCGTATGCAGACGATAAAGCTATCGTCGGTGGTATCGC  
10 CCGTCTCGATGGTCGTCCGGTGATGATCATTGGTCATCAAAAAGGTCGTGAAAC  
CAAAGAAAAAATTCGCCGTAACCTTGGTATGCCAGCGCCAGAAGGTTACCGCA  
AAGCACTGCGTCTGATGCAAATGGCTGAACGCTTTAAGATGCCTATCATCACCT  
TTATCGACACCCCGGGGGCTTATCCTGGCGTGGGCGCAGAAGAGCGTGGTCAG  
TCTGAAGCCATTGCACGCAACCTGCGTGAAATGTCTCGCCTCGGCGTACCGGTA  
15 GTTTGTACGGTTATCGGTGAAGGTGGTTCTGGCGGTGCGCTGGCGATTGGCGTG  
GGCGATAAAGTGAATATGCTGCAATACAGCACCTATTCCGTTATCTCGCCGGAA  
GGTTGTGCGTCCATTCTGTGGAAGAGCGCCGACAAAGCGCCGCTGGCGGCTGA  
AGCGATGGGTATCATTGCTCCGCGTCTGAAAGAACTGAAACTGATCGACTCCAT  
CATCCCGGAACCACTGGGTGGTGCTACCGTAACCCGGAAGCGATGGCGGCAT  
20 CGTTGAAAGCGCAACTGCTGGCGGATCTGGCCGATCTCGACGTGTTAAGCACTG  
AAGATTTAAAAAATCGTCGTTATCAGCGCCTGATGAGCTACGGTTACGCGTAA

**FIGURE 51**

SEQ ID NO: 65

MSLNFLDFEQPIAELEAKIDSLTAVSRQDEKLDINIDEEVHRLREKSVELTRK  
5 IFADLGAWQIAQLARHPQRPYTLDYVRLAFDEFDELADRAYADDKAIVGGIARL  
DGRPVMIIIGHQKGRETKEKIRRNFVGMPAPEGYRKALRLMQMAERFKMPIITFIDTP  
GAYPGVGAEERGQSEAIARNLREMSRLGVPVVCTVIGEGGSGGALAIGVGDKVN  
LQYSTYSVISPEGCASILWKSADKAPLAAEAMGIIAPRLKELKLIDSIIPEPLGGAHR  
NPEAMAASLKAQLLADLADLDVLSTEDLKNRRYQRLMSYGYA

**FIGURE 52**

SEQ ID NO: 66

ATGAGTCTGAATTTCTTGATTTTGAACAGCCGATTGCAGAGCTGGAAG  
5 CGAAAATCGATTCTCTGACTGCGGTTAGCCGTCAGGATGAGAACTGGATATTA  
ACATCGATGAAGAAGTGCATCGTCTGCGTGAAAAAAGCGTAGAACTGACACGT  
AAAATCTTCGCCGATCTCGGTGCATGGCAGATTGCGCAACTGGCACGCCATCCA  
CAGCGTCCTTATACCCTGGATTACGTTGCGCTGGCATTGATGAATTTGACGAA  
CTGGCTGGCGACCGCGCGTATGCAGACGATAAAGCTATCGTCGGTGGTATCGC  
10 CCGTCTCGATGGTCGTCCGGTGATGATCATTGGTCATCAAAAAGGTCGTGAAAC  
CAAAGAAAAAATTCGCCGTAACCTTTGGTATGCCAGCGCCAGAAGGTTACCGCA  
AAGCACTGCGTCTGATGCAAATGGCTGAACGCTTTAAGATGCCTATCATCACCT  
TTATCGACACCCCGGGGGCTTATCCTGGCGTGGGCGCAGAAGAGCGTGGTCAG  
TCTGAAGCCATTGCACGCAACCTGCGTGAAATGTCTCGCCTCGGCGTACCGGTA  
15 GTTTGTACGGTTATCGGTGAAGGTGGTTCTGGCGGTGCGCTGGCGATTGGCGTG  
GGCGATAAAGTGAATATGCTGCAATACAGCACCTATTCCGTTATCTCGCCGGAA  
GGTTGTGCGTCCATTCTGTGGAAGAGCGCCGACAAAGCGCCGCTGGCGGCTGA  
AGCGATGGGTATCATTGCTCCGCGTCTGAAAGAACTGAAACTGATCGACTCCAT  
CATCCCGGAACCACTGGGTGGTGCTCACCGTAACCCGGAAGCGATGGCGGCAT  
20 CGTTGAAAGCGCAACTGCTGGCGGATCTGGCCGATCTCGACGTGTTAAGCACTG  
AAGATTTAAAAAATCGTCGTTATCAGCGCCTGATGAGCTACGGTTACGCGTAA

**FIGURE 53**

SEQ ID NO: 67

MSLNFLDFEQPIAELEAKIDSLTAVSRQDEKLDINIDEEVHRLREKSVELTRK  
5 IFADLGAWQIAQLARHPQRPYTLDYVRLAFDEFDELADRAYADDKAIVGGIARL  
DGRPVMIIIGHQKGRETKEKIRRNFGMPAPEGYRKALRLMQMAERFKMPIITFIDTP  
GAYPGVGAEERGQSEAIARNLREMSRLGVPVVCTVIGEGGSGGALAIGVGDKVN  
LQYSTYSVISPEGCASILWKSADKAPLAAEAMGIIAPRLKELKLIDSIPEPLGGAHR  
NPEAMAASLKAQLLADLADLDVLSTEDLKNRRYQRLMSYGYA

10

**FIGURE 54**

SEQ ID NO: 68

Forward PCR Primer

5 GCGGCGGCCCATATGAGTCTGAATTCCTTGATTTG

SEQ ID NO: 69

10

Reverse PCR Primer

GCGCGGATCCATCAAATGCCAGGCGAACG

**FIGURE 55****TABLE 9 Properties of acetyl-CoA carboxylase carboxyl transferase subunit alpha from *E. coli***

TABLE 9 -- acetyl-CoA carboxylase carboxyl transferase subunit alpha from <i>E. coli</i> -- SEQ ID NO: 64-SEQ ID NO: 67	
Melting temperature (°C) of SEQ ID NO: 68 (forward PCR primer)	66
Restriction enzyme for SEQ ID NO: 68 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 69 (reverse PCR primer)	58
Restriction enzyme for SEQ ID NO: 69 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 64	960
Number of amino acid residues in SEQ ID NO: 65	319
Number of different nucleic acid residues between SEQ ID NO: 64 and SEQ ID NO: 66	0
Number of different amino acid residues between SEQ ID NO: 65 and SEQ ID NO: 67	0
Calculated molecular weight of SEQ ID NO: 65 polypeptide (kDa)	35.2
Calculated pI of SEQ ID NO: 65 polypeptide	5.6
Solubility of SEQ ID NO: 67 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Less than one third
Solubility of SEQ ID NO: 67 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	Approaching one third
Amount of purified polypeptide having SEQ ID NO: 67, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	20.5
Amount of purified <sup>15</sup> N labeled polypeptide having SEQ ID NO: 67, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	9.4
Amount of purified polypeptide having SEQ ID NO: 67 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	55.4
Amount of purified <sup>15</sup> N labeled polypeptide having SEQ ID NO: 67 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	6.7
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. No interacting proteins were observed by using at least one of the methods described in those examples.	

**FIGURE 56****TABLE 10 Bioinformatic Analyses of acetyl-CoA carboxylase carboxyl transferase subunit alpha from *E. coli***

TABLE 10 -- acetyl-CoA carboxylase carboxyl transferase subunit alpha from <i>E. coli</i> -- SEQ ID NO: 64-SEQ ID NO: 67	
COG Category	Lipid metabolism
COG ID Number	COG0825
Is SEQ ID NO: 65 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 65	None
Source organism for closest PDB protein to SEQ ID NO: 65	N/A
e-value for closest PDB Protein to SEQ ID NO: 65	N/A
% Identity between SEQ ID NO: 65 and the closest protein from PDB	N/A
% Positives between SEQ ID NO: 65 and the closest protein from PDB	N/A
Number of Protein Hits in the VGDB to SEQ ID NO: 65	11
Number of Microorganisms having VGDB Hits to SEQ ID NO: 65	10
Microorganisms having VGDB Hits to SEQ ID NO: 65 <sup>1</sup>	[paer][nmen][hinf][ecoli][ctra] [saur][bsub][hpyl][efae][spne]
First predicted epitopic region of SEQ ID NO: 65: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 70 :RLGVPVVCTVI, 1.274, 190->200
Second predicted epitopic region of SEQ ID NO: 65: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 71 :AASLKAQLLAD- LADLDVLSTE, 1.133, 283->303
Third predicted epitopic region of SEQ ID NO: 65: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 72 :FADLGAWQIAQ- LARHPQRPYTLDYVRLAFD, 1.121, 55->84

- 5 <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Eschericia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpax* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.



**FIGURE 57**

SEQ ID NO: 73

ATGTTAGATTTTGAAAAACCACTTTTGAAATTCGAAATAAAATTGAATC  
5 TTTAAAAGAATCTCAAGATAAAAATGATGTGGATTTACAAGAAGAAATTGACA  
TGCTTGAAGCGTCATTGGAACGAGAACTAAAAAATATATACAAATCTAAAA  
CCATGGGATCGTGTGCAAATTGCGCGTTTGCAAGAAAGACCTACGACCCTAGA  
TTATATTCCATATATCTTTGATTCGTTTATGGAACCTACATGGTGATCGTAATTTT  
AGAGATGATCCAGCAATGATTGGTGGTATTGGCTTTTTAAATGGTCGTGCTGTT  
10 ACAGTTATTGGACAACAACGTGGAAAAGATACAAAAGATAATATTTATCGAAA  
TTTTGGTATGGCGCATCCAGAAGGTTATCGAAAAGCATTACGTTTAATGAAACA  
AGCTGAAAAATTCAATCGTCCTATCTTTACATTTATAGATACAAAAGGTGCATA  
TCCTGGTAAAGCTGCTGAAGAACGTGGACAAAGTGAATCTATCGCAACAAATT  
TGATTGAGATGGCTTCATTAAGTACCAGTTATTGCGATTGTCATTGGTGAAG  
15 GTGGCAGTGGAGGTGCTCTAGGTATTGGTATTGCCAATAAAGTATTGATGTTAG  
AGAATAGTACTTACTCTGTTATATCTCCTGAAGGTGCAGCGGCATTATTATGGA  
AAGACAGTAATTTGGCTAAAATTGCAGCTGAAACAATGAAAATTACTGCCCAT  
GATATTAAGCAATTAGGTATTATAGATGATGTCATTTCTGAACCACTTGGCGGT  
GCACATAAAGATATTGAACAGCAAGCTTTAGCTATTAAATCAGCGTTTGTTGCA  
20 CAGTTAGATTCACTTGAGTCATTATCACGTGATGAAATTGCTAATGATCGCTTT  
GAAAAATTCAGAAATATCGGTTCTTATATAGAATAA

**FIGURE 58**

SEQ ID NO: 74

MLDFEKPLFEIRNKIESLKESQDKNDVDLQEEIDMLEASLERETKKIYTNLKP  
5 WDRVQIARLQERPTTLDYIPYIFDSFMELHGDRNFRDDPAMIGGIGFLNGRAVTVIG  
QQRGKDTKDNIYRNFGMAHPEGYRKALRLMKQAEKFNRPIFTFIDTKGAYPGKAA  
EERGQSESIATNLIEMASLKVPVIAIVIGEGGSGGALGIGIANKVLMLNSTYSVISPE  
GAAALLWKDSNLAKIAAETMKITAHDIKQLGIIDDVISEPLGGAHKDIEQQALAIKS  
AFVAQLDSLESLSRDEIANDRFKFRNIGSYIE

**FIGURE 59**

SEQ ID NO: 75

ATGTTAGATTTTGAAAAACCACTTTTGTGAAATTCGAAATAAAATTGAATC  
5 TTTAAAAGAATCTCAAGATAAAAAATGATGTGGATTTACAAGAAGAAATTGACA  
TGCTTGAAGCGTCATTGGAACGAGAACTAAAAAATATATACAAATCTAAAA  
CCATGGGATCGTGTGCAAATTGCGCGTTTGCAAGAAAGACCTACGACCCTAGA  
TTATATTCCATATATCTTTGATTCGTTTATGGAACCTACATGGTGATCGTAATTTT  
AGAGATGATCCAGTAATGATTGGTGGTATTGGCTTTTTAAATGGTCGTGCTGTT  
10 ACAGTTATTGGACAACAACGTGGAAAAGATACAAAAGATAATTTTATCGAAA  
TTTTGGTATGGCGCATCCAGAAGGTTATCGAAAAGCATTACGTTTAATGAAACA  
AGCTGAAAAATTCAATCGTCCTATCTTTACATTTATAGATACAAAAGGTGCATA  
TCCTGGTAAAGCTGCTGAAGAACGTGGACAAAGTGAATCTATCGCAACAAATT  
TGATTGAGATGGCTTCATTAAGTACCAGTTATTGCGATTGTCATTGGTGAAG  
15 GTGGCAGTGGAGGTGCTCTAGGTATTGGTATTGCCAATAAAGTATTGATGTTAG  
AGAATAGTACTTACTCTGTTATATCTCCTGAAGGTGCAGCGGCATTATTATGGA  
AAGACAGTAATTTGGCTAAAATTGCAGCTGAAACAATGAAAATTACTGCCCAT  
GATATTAAGCAATTAGGTATTATAGATGATGTCATTTCTGAACCACTTGGCGGT  
GCACATAAAGATATTGAACAGCAAGCTTTAGCTATTAAATCAGCGTTTGTTGCA  
20 CAGTTAGATTCACTTGAGTCATTATCACGTGATGAAATTGCTAATGATCGCTTT  
GAAAAATTCAGAAATATCGGTTCTTATATAGAATAA

**FIGURE 60**

SEQ ID NO: 76

MLDFEKPLFEIRNKIESLKESQDKNDVDLQEEIDMLEASLERETKKIYTNLKP  
5 WDRVQIARLQERPTTLDYIPYIFDSFMELHGDRNFRDDPVMIGGIGFLNGRAVTVIG  
QQRGKDTKDNIYRNFGMAHPEGYRKALRLMKQAEKFNRPIFTFIDTKGAYPGKAA  
EERGQSESIATNLIEMASLKVPVIAIVIGEGGSGGALGIGIANKVLMLNSTYSVISPE  
GAAALLWKDSNLAKIAAETMKITAHDIKQLGIIDDVISEPLGGAHKDIEQQALAIKS  
AFVAQLDSLESLSRDEIANDRFEKFRNIGSYIE

**FIGURE 61**

SEQ ID NO: 77

Forward PCR Primer

5 GCGGCGGCCCATATGTTAGATTTTGAAAAACCACTTTTGTG

SEQ ID NO: 78

10

GCGCGGATCCACCATGTAGTTCCATAAACGAATC

**FIGURE 62****TABLE 11 Properties of acetyl-CoA carboxylase carboxyl transferase subunit alpha from *S. aureus***

TABLE 11 -- acetyl-CoA carboxylase carboxyl transferase subunit alpha from <i>S. aureus</i> - - SEQ ID NO: 73-SEQ ID NO: 76	
Melting temperature (°C) of SEQ ID NO: 77 (forward PCR primer)	70
Restriction enzyme for SEQ ID NO: 77 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 78 (reverse PCR primer)	66
Restriction enzyme for SEQ ID NO: 78 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 73	845
Number of amino acid residues in SEQ ID NO: 74	314
Number of different nucleic acid residues between SEQ ID NO: 73 and SEQ ID NO: 75	1
Number of different amino acid residues between SEQ ID NO: 74 and SEQ ID NO: 76	1
Calculated molecular weight of SEQ ID NO: 74 polypeptide (kDa)	35.1
Calculated pI of SEQ ID NO: 74 polypeptide	4.9
Solubility of SEQ ID NO: 76 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching 100%
Amount of purified polypeptide having SEQ ID NO: 76, prepared and purified as described in the Exem The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	4.14
Amount of purified <sup>15</sup> N labeled polypeptide having SEQ ID NO: 76, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	22.30
Amount of purified polypeptide having SEQ ID NO: 76 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	13.80
Amount of purified <sup>15</sup> N labeled polypeptide having SEQ ID NO: 76 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	58.80

**FIGURE 62-B**

TABLE 11 -- acetyl-CoA carboxylase carboxyl transferase subunit alpha from <i>S. aureus</i> - - SEQ ID NO: 73-SEQ ID NO: 76	
Z-score for the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 76, determined as described in EXAMPLE 9	8.6E-03
Number of matched peptides in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 76, determined as described in EXAMPLE 9	8
Minimum sequence coverage in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 76, determined as described in EXAMPLE 9	21
Calculated molecular weight of SEQ ID NO: 74 polypeptide (Da), determined as described in EXAMPLE 10	37523
Experimental molecular weight of SEQ ID NO: 76 polypeptide (Da), determined as described in EXAMPLE 10	37646
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. The identity of an interacting protein identified by using at least one of the methods described in those examples is: malate:quinone oxidoreductase (gi 13702564)	

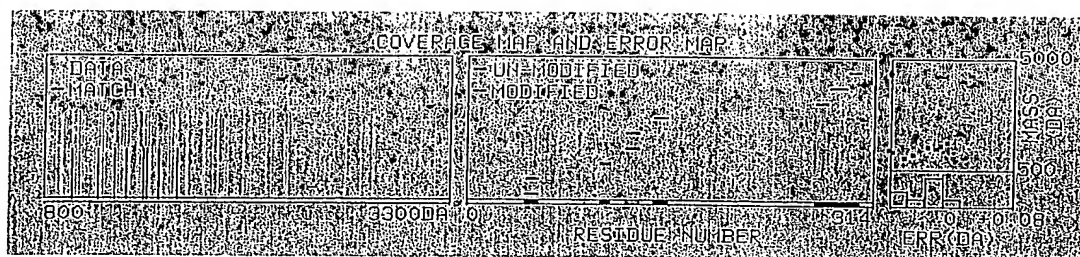
**FIGURE 63****TABLE 12 Bioinformatic Analyses of acetyl-CoA carboxylase carboxyl transferase subunit alpha from *S. aureus***

TABLE 12 -- acetyl-CoA carboxylase carboxyl transferase subunit alpha from <i>S. aureus</i> -- SEQ ID NO: 73-SEQ ID NO: 76	
COG Category	Lipid metabolism
COG ID Number	COG0825
Is SEQ ID NO: 74 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 74	None
Source organism for closest PDB protein to SEQ ID NO: 74	N/A
e-value for closest PDB Protein to SEQ ID NO: 74	N/A
% Identity between SEQ ID NO: 74 and the closest protein from PDB	N/A
% Positives between SEQ ID NO: 74 and the closest protein from PDB	N/A
Number of Protein Hits in the VGDB to SEQ ID NO: 74	11
Number of Microorganisms having VGDB Hits to SEQ ID NO: 74	10
Microorganisms having VGDB Hits to SEQ ID NO: 74 <sup>1</sup>	[paer][nmen][hinf][ecoli][ctra] [saur][bsub][hpyl][efae][spne]
First predicted epitopic region of SEQ ID NO: 74: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 79 :MASLKVPVIAIVIGE, 1.226,181->195
Second predicted epitopic region of SEQ ID NO: 74: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 80 :QQALAIKSAFV-AQLDSLESL, 1.126,274->293
Third predicted epitopic region of SEQ ID NO: 74: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 81 :TLDYIPYIFD, 1.115,68->77

- 5                   <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Eschericia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpax* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.



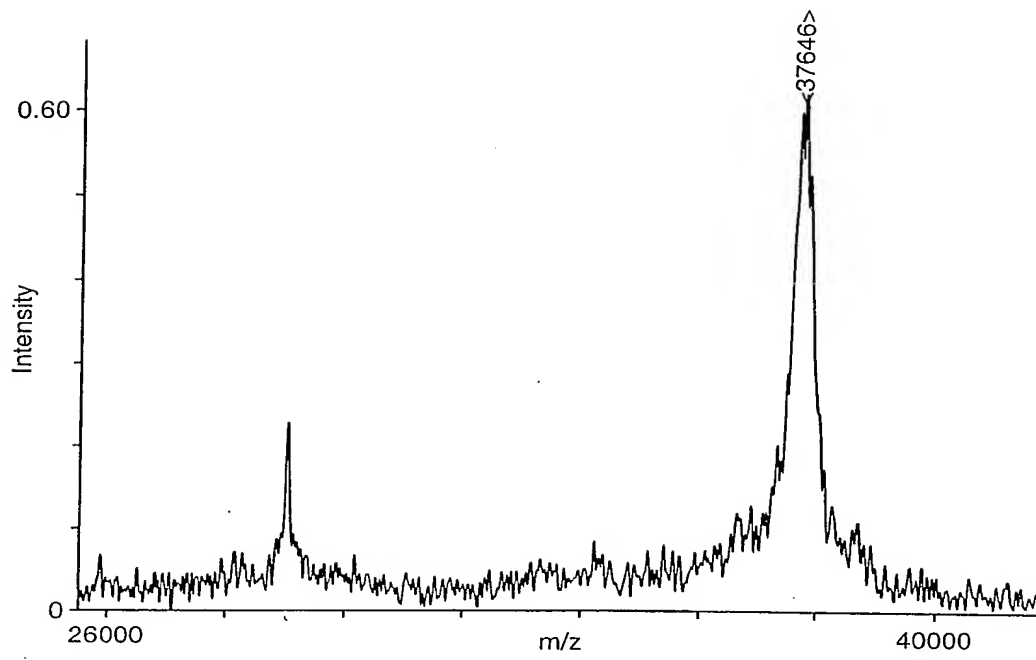
FIGURE 64



Note: click on the ☐ symbol to change column format.

Measured Mass (M)	Avg/ Mono	Computed Mass	Error (Da)	Residues Start	Residues To	Missed Cut	Peptide sequence
970.496	M	970.555	-0.060	105	113	0	AVTVIGQQR
1127.570	M	1127.618	-0.048	271	280	0	DIEQQALAIK
1235.505	M	1235.578	-0.073	296	305	1	DEIANDRF EK
1304.610	M	1304.687	-0.077	47	56	0	IYTNLKPWDR
1405.638	M	1405.656	-0.018	124	135	1	NFGMAHPEG YRK
1405.638	M	1405.656	-0.018	124	135	1	NFGMAHPEG YRK
1432.753	M	1432.782	-0.029	46	56	1	KIYTNLKPWDR
1497.784	M	1497.797	-0.014	146	157	0	FNRPIFTFIDTK
1621.824	M	1621.830	-0.006	281	295	0	SAFVAQLDSLESLSR

FIGURE 65



**FIGURE 66**

SEQ ID NO: 82

ATGGGAAAATATTTTGGTACAGACGGAGTAAGAGGTGTCGCAAACCAA  
5 GAACTAACACCTGAATTGGCATTAAATTAGGAAGATACGGTGGCTATGTTCTA  
GCACATAATAAAGGTGAAAAACACCCACGTGTACTTGTAGGTGCGGATACTAG  
AGTTTCAGGTGAAATGTTAGAATCAGCATTAAATAGCTGGTTTGATTTC AATTGG  
TGCAGAAGTGATGCGATTAGGTATTATTTCAACACCAGGTGTTGCATATTTAAC  
ACGCGATATGGGTGCAGAGTTAGGTGTAATGATTTCAGCCTCTCATAATCCAGT  
10 TGCAGATAATGGTATTAAATTCTTTGGATCAGATGGTTTTAACTATCAGATGA  
ACAAGAAAATGAAATTGAAGCATTATTGGATCAAGAAAACCCAGAATTACCAA  
GACCAGTTGGCAATGATATTGTACATTATTCAGATTACTTTGAAGGGGCACAAA  
AATATTTGAGCTATTTAAAATCAACAGTAGATGTAACTTTGAAGGTTTGAAAA  
TTGCTTTAGATGGTGCAAATGGTTCAACATCATCACTAGCGCCATTCTTATTTGG  
15 TGACTTAGAAGCAGATACTGAAACAATTGGATGTAGTCCTGATGGATATAATAT  
CAATGAGAAATGTGGCTCTACACATCCTGAAAAATTAGCTGAAAAAGTAGTTG  
AAACTGAAAGTGATTTTGGGTAGCATTTGACGGCGATGGAGACAGAATCATA  
GCAGTAGATGAGAATGGTCAAATCGTTGACGGTGACCAAATTATGTTTATTATT  
GGTCAAGAAATGCATAAAAATCAAGAATTGAATAATGACATGATTGTTTCTACT  
20 GTTATGAGTAATTTAGGTTTTTACAAAGCGCTTGAACAAGAAGGAATTAAATCT  
AATAAACTAAAGTTGGCGACAGATATGTAGTAGAAGAAATGCGTCGCGGTAA  
TTATAACTTAGGTGGAGAACAATCTGGACATATCGTTATGATGGATTACAATAC  
AACTGGTGATGGTTTATTA ACTGGTATTCAATTAGCTTCTGTAATAAAAATGAC  
TGGTAAATCACTAAGTGAATTAGCTGGACAAATGAAAAAATATCCACAATCAT  
25 TAATTAACGTACGCGTAACAGATAAATATCGTGTTGAAGAAAATGTTGACGTTA  
AAGAAGTTATGACTAAAGTAGAAGTAGAAATGAATGGAGAAGGTGGAATTTTA  
GTAAGACCTTCTGGAACAGAACCATTAGTTCGTGTCATGGTTGAAGCAGCAACT  
GATGAAGATGCTGAAAGATTTGCACAACAAATAGCTGATGTGGTTCAAGATAA  
AATGGGATTAGATAAAATAA

**FIGURE 67**

SEQ ID NO: 83

MGKYFGTDGVRGVANQELTPELAFKLGRYGGYVLAHNKGEKHPRVLVGR  
5 DTRVSGEMLESALIAGLISIGAEVMRLGIISTPGVAYLTRDMGAELGVMISASHNPV  
ADNGIKFFGSDGFKLSDEQENEIEALLDQENPELPRPVGNDIVHYSDYFEGAQKYLS  
YLKSTVDVNFEGLKIALDGANGSTSSLAPFLFGDLEADTETIGCSPDGYNINEKCGS  
THPEKLAEKVVETESDFGLAFDGDGDRIIAVDENGQIVDGDQIMFIIGQEMHKNQE  
LNNDMIVSTVMSNLGFYKALEQEGIKSNKTKVGDRYVVEEMRRGNYNLGGEQSG  
10 HIVMMDYNTTGDGLLTGIQLASVIKMTGKSLSELAGQMKKYPQSLINVRVTDKYR  
VEENVDVKEVMTKVEVEMNGEGRILVRPSGTEPLVRVMVEAATDEDAERFAQQIA  
DVVQDKMGLDK

**FIGURE 68**

SEQ ID NO: 84

ATGGGAAAATATTTTGGTACAGACGGAGTAAGAGGTGTCGCAAACCAA  
5 GAACTAACACCTGAATTGGCATTAAATTAGGAAGATACGGTGGCTATGTTCTA  
GCACATAATAAAGGTGAAAAACACCCACGTGTACTTGTAGGTTCGCGATACTAG  
AGTTTCAGGTGAAATGTTAGAATCAGCATTAAATAGCTGGTTTGATTTC AATTGG  
TGCAGAAGTGATGCGATTAGGTATTATTTCAACACCAGGTGTTGCATATTTAAC  
ACGCGATATGGGTGCAGAGTTAGGTGTAATGATTT CAGCCTCTCATAATCCAGT  
10 TGCAGATAATGGTATTAAATTCTTTGGATCAGATGGTTTTAAACTATCAGATGA  
ACAAGAAAATGAAATTGAAGCATTATTGGATCAAGAAAACCCAGAATTACCAA  
GACCAGTTGGCAATGATATTGTACATTATTCAGATTACTTTGAAGGGGCACAAA  
AATATTTGAGCTATTTAAAATCAACAGTAGATGT TAACTTTGAAGGTTTGAAAA  
TTGCTTTAGATGGTGCAAATGGTTCAACATCATCACTAGCGCCATTCTTATTTGG  
15 TGACTTAGAAGCAGATACTGAAACAATTGGATGTAGTCCTGATGGATATAATAT  
CAATGAGAAATGTGGCTCTACACATCCTGAAAAATTAGCTGAAAAAGTAGTTG  
AAACTGAAAGTGATTTTGGGTTAGCATTTGACGGCGATGGAGACAGAATCATA  
GCAGTAGATGAGAATGGTCAAATCGTTGACGGTGACCAAATTATGTTTATTATT  
GGTCAAGAAATGCATAAAAATCAAGAATTGAATAATGACATGATTGTTTCTACT  
20 GTTATGAGTAATTTAGGTTTTTACAAAGCGCTTGAACAAGAAGGAATTAAATCT  
AATAAAACTAAAGTTGGCGACAGATATGTAGTAGAAGAAATGCGTCGCGGTAA  
TTATAACTTAGGTGGAGAACAATCTGGACATATCGTTATGATGGATTACAATAC  
AACTGGTGATGGTTTATTA ACTGGTATTCAATTAGCTTCTGTAATAAAAATGAC  
TGGTAAATCACTAAGTGAATTAGCTGGACAAATGAAAAAATATCCACAATCAT  
25 TAATTAACGTACGCGTAACAGATAAATATCGTGTTGAAGAAAATGTTGACGTTA  
AAGAAGTTATGACTAAAGTAGAAGTAGAAATGAATGGAGAAGGTCGAATTTTA  
GTAAGACCTTCTGGAACAGAACCATTAGTTCGTGTCATGGTTGAAGCAGCAACT  
GATGAAGATGCTGAAAGATTTGCACAACAAATAGCTGATGTGGTTCAAGATAA  
AATGGGATTAGATAAATAA

**FIGURE 69**

SEQ ID NO: 85

MGKYFGTDGVRGVANQELTPELAFKLGRYGGYVLAHNKGEKHPRVLVGR  
5 DTRVSGEMLESALIAGLISIGAEVMRLGIISTPGVAYLTRDMGAELGVMISASHNPV  
ADNGIKFFGSDGFKLSDEQENEIEALLDQENPELPRPVGNDIVHYSDFEGAQKYL  
YLKSTVDVNFEGLKIALDGANGSTSSLAPFLFGDLEADTETIGCSPDGYNINEKCGS  
THPEKLAEKVVETESDFGLAFDGDGDRIIAVDENGQIVDGDQIMFIIGQEMHKNQE  
LNNDMIVSTVMSNLGFYKALEQEGIKSNKTKVGDYVVEEMRRGNYNLGGEQSG  
10 HIVMMDYNTTGDGLLTGIQLASVIKMTGKSLSELAGQMKKYPQSLINVRVTDKYR  
VEENVDVKEVMTKVEVEMNGEGRILVRPSGTEPLVRVMVEAATDEDAERFAQQIA  
DVVQDKMGLDK

**FIGURE 70**

SEQ ID NO: 86

Forward PCR Primer

5 GCGGCGGCCCATATGGGAAAATATTTTGGTACAG

SEQ ID NO: 87

10

Reverse PCR Primer

GCGCGGATCCAACACCTGGTGTTGAAATAATAC

**FIGURE 71****TABLE 13 Properties of phosphoglucosamine-mutase from *S. aureus***

TABLE 13 -- phosphoglucosamine-mutase from <i>S. aureus</i> -- SEQ ID NO: 82-SEQ ID NO: 85	
Melting temperature (°C) of SEQ ID NO: 86 (forward PCR primer)	58
Restriction enzyme for SEQ ID NO: 86 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 87 (reverse PCR primer)	62
Restriction enzyme for SEQ ID NO: 87 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 82	1356
Number of amino acid residues in SEQ ID NO: 83	451
Number of different nucleic acid residues between SEQ ID NO: 82 and SEQ ID NO: 84	0
Number of different amino acid residues between SEQ ID NO: 83 and SEQ ID NO: 85	0
Calculated molecular weight of SEQ ID NO: 83 polypeptide (kDa)	49.3
Calculated pI of SEQ ID NO: 83 polypeptide	4.4
Solubility of SEQ ID NO: 85 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching 100%
Amount of purified polypeptide having SEQ ID NO: 85, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	2.8
Amount of purified polypeptide having SEQ ID NO: 85 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	8.0
Z-score for the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 85, determined as described in EXAMPLE 9	4E-07
Number of matched peptides in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 85, determined as described in EXAMPLE 9	16
Minimum sequence coverage in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 85, determined as described in EXAMPLE 9	36
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. No interacting proteins were observed by using at least one of the methods described in those examples.	

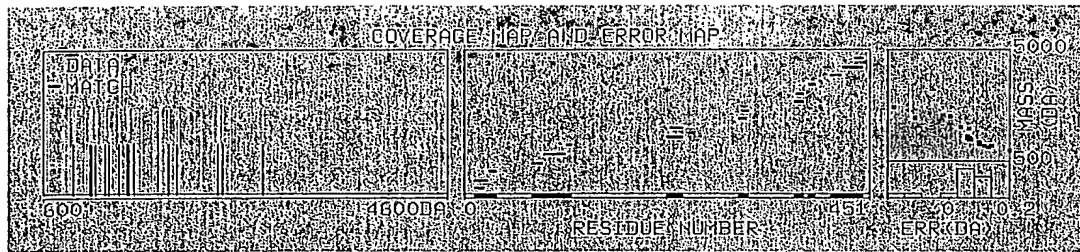


**FIGURE 72****TABLE 14 Bioinformatic Analyses of phosphoglucosamine-mutase from *S. aureus***

TABLE 14 -- phosphoglucosamine-mutase from <i>S. aureus</i> -- SEQ ID NO: 82-SEQ ID NO: 85	
COG Category	Carbohydrate transport and metabolism
COG ID Number	COG1109
Is SEQ ID NO: 83 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 83	Alpha-D-Glucose-1,6-Bisphosphate, (3pmg)
Source organism for closest PDB protein to SEQ ID NO: 83	<i>Oryctolagus cuniculus</i>
e-value for closest PDB Protein to SEQ ID NO: 83	8E-09
% Identity between SEQ ID NO: 83 and the closest protein from PDB	27
% Positives between SEQ ID NO: 83 and the closest protein from PDB	43
Number of Protein Hits in the VGDB to SEQ ID NO: 83	13
Number of Microorganisms having VGDB Hits to SEQ ID NO: 83	10
Microorganisms having VGDB Hits to SEQ ID NO: 83 <sup>1</sup>	[saur][bsub][spne][paer][ctra][nmen][ecoli][hinf][hpyl][efae]
First predicted epitopic region of SEQ ID NO: 83: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 88 :TEPLVRVMVE, 1.166,416->425
Second predicted epitopic region of SEQ ID NO: 83: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 89 :LTGIQLASVIKM, 1.147,345->356
Third predicted epitopic region of SEQ ID NO: 83: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 90 :HPRVLVGRD, 1.141,42->50

- <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Eschericia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpax* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

FIGURE 73



Note: click on the ☐ symbol to change column format.

Measured Mass (M)	Avg/ Mono	Computed Mass	Error (Da)	<input type="checkbox"/> Residues	Missed	Cut	Peptide sequence
903.610	H	903.412	0.198	113	120	0	FFGSDGFK
1080.666	H	1080.538	0.128	312	319	1	YVVEEMRR
1088.739	H	1088.597	0.141	371	379	0	YPOS LINVR
1120.685	H	1120.566	0.119	29	38	0	YGGYVLAH NK
1216.795	H	1216.692	0.103	370	379	1	KYFQSLINVR
1249.733	H	1249.630	0.103	384	393	1	YRVEENV DVK
1351.725	H	1351.655	0.070	308	318	1	VGDRYVVEEMR
1360.774	H	1360.698	0.076	435	446	0	FAQQIADV VQDK
1435.912	H	1435.850	0.062	409	421	0	ILVRPSGTEPLVR
1459.907	H	1459.839	0.068	76	89	0	LGIISTPGVAYLTR
1515.856	H	1515.792	0.064	12	25	0	GVANQELTPELAFK
1842.066	H	1841.999	0.068	12	28	1	GVANQELTPELAFKLGR
1927.884	H	1927.843	0.041	230	247	0	VVETESDFGLAFDGDGDR
2325.000	H	2325.108	-0.108	90	112	0	DHGAELGVHISASHNPVADNGIK
2369.110	H	2369.101	0.009	226	247	1	LAEKVVETESDFGLAFDGDGDR
2777.142	H	2777.317	-0.175	422	446	1	VMVEAATDEDAERFAQQIADV VQDK

**FIGURE 74**

SEQ ID NO: 91

ATGAAACAAACGATTATTCTTTTATATGGTGGACGGAGTGCGGAACGCG  
5 AAGTCTCTGTCCTTTTCAGCTGAGAGTGTCATGCGTGCGGTCGATTACGACCGTT  
TCACAGTCAAGACTTTCTTTATCAGTCAGTCAGGTGACTTTATCAAAACACAGG  
AATTTAGTCATGCTCCGGGGCAAGAAGACCGTCTCATGACCAATGAAACCATT  
GATTGGGATAAGAAAGTTGCACCAAGTGCTATCTACGAAGAAGGTGCAGTGGT  
CTTTCCAGTCCTTCACGGGGCCAATGGGAGAAGATGGCTCTGTTCAAGGATTCTT  
10 GGAAGTTTTGAAAATGCCTTACGTTGGTTGCAACATTTTGTCAAGTCTTGC  
CATGGATAAAATCACGACTAAGCGTGTTCTGGAATCTGCTGGTATTGCCCAAGT  
TCCTTATGTGGCTATCGTTGAAGGCGATGATGTGACTGCTAAAATCGCTGAAGT  
GGAAGAAAAATTGGCTTATCCAGTCTTCACTAAGCCGTCAAACATGGGGTCTA  
GTGTCGGTATTTCTAAGTCTGAAAACCAAGAAGAACTCCGTCAAGCCTTAAAC  
15 TTGCCTTCCGATATGACAGCCGTGTCTTGGTTGAGCAAGGAGTGAATGCCCGTG  
AAATTGAGGTTGGCCTCTTGGGTAACTACGATGTCAAGAGCACGCTACCAGGA  
GAAGTTGTCAAGGACGTTGCCTTTTATGACTACGATGCCAAGTATATTGATAAC  
AATATTACTATGGATATTCCTGCCAAAATCAGTGATGATGTGGTGGCTGTCATG  
CGTCAAAATGCAGAAACAGCCTTCCGTGCCATTGGTGGCCTTGGTCTATCTCGT  
20 TGCGATTTCTTCTATACAGATAAGGGAGAGATTTTCTCAACGAGCTCAATACT  
ATGCCAGGTTTCACCCAGTGGTCTATGTACCCACTACTTTGGGACAATATGGGG  
ATCAGCTACCCAAAATAATCGAGCGTTTGGTTGACCTTGCCAAGGAAAGTTTT  
GACAAGCGCGAAGCGCATTTGATATAA

**FIGURE 75**

SEQ ID NO: 92

          MKQTIILLYGGRSAEREVSVLSAESVMRAVDYDRFTVKTFFISQSGDFIKTQ  
5  EFSHAPGQEDRLMTNETIDWDKKVAPSAIYEEGAVVFPVLHGPMGEDGSVQGFLE  
      VLKMPYVGCNLSLSSSLAMDKITTKRVLESAGIAQVPYVAIVEGDDVTAKIAEVEEK  
      LAYPVFTKPSNMGSSVGISKSENQEELRQALKLAFRYDSRVLVEQGVNAREIEVGL  
      LGNYDVKSTLPGEVVKDVAFYDYDAKYIDNNITMDIPAKISDDVVAVMRQNAETA  
      FRAIGGLGLSRCDDFFYTDKGEIFLNELNTMPGFTQWSMYPLLWDNMGISYPKLIER  
10  LVDLAKESFDKREAHLI-

**FIGURE 76**

SEQ ID NO: 93

ATGAAACAAACGATTATTCTTTTATATGGTGGACGGAGTGCGGAACGCG  
5 AAGTCTCTGTCCTTTTCAGCTGAGAGTGTCATGCGTGCGGTCAATTACGACCGTT  
TCACAGTCAAGACTTTCTTTATCAGTCAGTCAGGTGACTTTATCAAAACACAGG  
AATTTAGTCATGCTCCGGGGCAAGAAGACCGTCTCATGACCAATGAAACCATT  
GATTGGGATAAGAAAGTTGCACCAAGTGCTATCTACGAAGAAGGTGCAGTGGT  
CTTTCCAGTCCTTCACGGGCCAATGGGAGAAGATGGCTCTGTTCAAGGATTCTT  
10 GGAAGTTTTGAAAATGCCTTACGTTGGTTGCAACATTTTGTTCATCAAGTCTTGC  
CATGGATAAAATCACGACTAAGCGTGTTCTGGAATCTGCTGGTATTGCCCAAGT  
TCCTTATGTGGCTATCGTTGAAGGCGATGATGTGACTGCTAAAATCGCTGAAGT  
GGAAGAAAAATTGGCTTATCCAGTCTTCATTAAGCCGTCAAACATGGGGTCTAG  
TGTCGGTATTTCTAAGTCTGAAAACCAAGAAGAACTCCGTCAAGCCTTAAAACT  
15 TGCCTTCCGATATGACAGCCGTGTCTTGGTTGAGCAAGGAGTGAATGCCCGTGA  
AATTGAGGTTGGCCTCTTGGGTAACACGATGTCAAGAGCACGCTACCTGGAG  
AAGTTGTCAAGGACGTTGCCTTTTATGACTACGATGCCAAGTATATTGATAACA  
AGATTACTATGGATATTCCTACCAAAATCAGTGATGATGTGGTGGCTGTCATGC  
GTCAAAATGCAGAAACAGCCTTCCGTGCCATTGGTGGCCTTGGTCTATCTCGTT  
20 GCGATTTCTTCTATACAGATAAGGGAGAGATTTTCTCAACGAGCTCAATACCA  
TGCCAGGTTTCACCCAGTGGTCTATGTACCCACTACTTTGGGACAATATGGGGA  
TCAGCTACCCAGAACTAATCGAGCGTTTGGTTGACCTTGCCAAGGAAAGTTTTG  
ACAAGCGCGAAGCGCATTGATATAA

**FIGURE 77**

SEQ ID NO: 94

          MKQTIILLYGGRSAEREVSVLSAESVMRAVNYDRFTVKTFFISQSGDFIKTQ  
5  EFSHAPGQEDRLMTNETIDWDKKVAPSAIYEEGAVVFPVLHGPMGEDGGSVQGFLE  
      VLKMPYVGCNLSLSSSLAMDKITTKRVLESAGIAQVPYVAIVEGDDVTAKIAEVEEK  
      LAYPVFIKPSNMGSSVGISKSENQEELRQALKLAFRYDSRVLVEQGVNAREIEVGLL  
      GNYDVKSTLPGEVVKDVAFYDYDAKYIDNKITMDIPTKISDDVVAVMRQNAETAF  
      RAIGGLGLSRCDFFYTDKGEIFLNELNTMPGFTQWSMYPLLWDNMGISYPELIERL  
10 VDLAKESFDKREAHLI

**FIGURE 78**

SEQ ID NO: 95

Forward PCR Primer

5 GCGGCGGCCCATATGAAACAAACGATTATTCTTTTATATG

SEQ ID NO: 96

10

Reverse PCR Primer

GCGCGGATCCTATCAAATGCGCTTCGCGC

**FIGURE 79****TABLE 15 Properties of D-alanine-D-alanine ligase A from *S. pneumoniae***

TABLE 15 -- D-alanine-D-alanine ligase A from <i>S. pneumoniae</i> -- SEQ ID NO: 91-SEQ ID NO: 94	
Melting temperature (°C) of SEQ ID NO: 95 (forward PCR primer)	68
Restriction enzyme for SEQ ID NO: 95 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 96 (reverse PCR primer)	58
Restriction enzyme for SEQ ID NO: 96 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 91	1044
Number of amino acid residues in SEQ ID NO: 92	347
Number of different nucleic acid residues between SEQ ID NO: 91 and SEQ ID NO: 93	7
Number of different amino acid residues between SEQ ID NO: 92 and SEQ ID NO: 94	5
Calculated molecular weight of SEQ ID NO: 92 polypeptide (kDa)	38.7
Calculated pI of SEQ ID NO: 92 polypeptide	4.5
Solubility of SEQ ID NO: 94 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one third
Solubility of SEQ ID NO: 94 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	Approximately two thirds
Amount of purified selmet labeled polypeptide having SEQ ID NO: 94, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 3 at the C-terminus as described in EXAMPLE 6.	5.4
Amount of purified selmet labeled polypeptide having SEQ ID NO: 94 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	16.0
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. No interacting proteins were observed by using at least one of the methods described in those examples.	
Crystals of a selenomethionine-substituted polypeptide having the sequence of SEQ ID NO: 94, prepared and purified as described above and having a His tag, are obtained using the following conditions: 2.5M ammonium sulfate, sodium acetate pH 4.5, 0.2 M lithium sulfate. The crystals were prepared using the following method: 20°C, sitting drop, 15 mg polypeptide per ml of solution.	



**FIGURE 80****TABLE 16 Bioinformatic Analyses of D-alanine-D-alanine ligase A from *S. pneumoniae***

TABLE 16 -- D-alanine-D-alanine ligase A from <i>S. pneumoniae</i> -- SEQ ID NO: 91-SEQ ID NO: 94	
COG Category	Cell envelope biogenesis, outer membrane
COG ID Number	COG1181
Is SEQ ID NO: 92 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 92	D-Alanine: D-Lactate Ligase (1ehi)
Source organism for closest PDB protein to SEQ ID NO: 92	<i>Leuconostoc Mesenteroides</i>
e-value for closest PDB Protein to SEQ ID NO: 92	7E-51
% Identity between SEQ ID NO: 92 and the closest protein from PDB	33
% Positives between SEQ ID NO: 92 and the closest protein from PDB	54
Number of Protein Hits in the VGDB to SEQ ID NO: 92	11
Number of Microorganisms having VGDB Hits to SEQ ID NO: 92	9
Microorganisms having VGDB Hits to SEQ ID NO: 92 <sup>1</sup>	[spne][saur][bsub][efae] [ecoli][hinf][bbur][paer]
First predicted epitopic region of SEQ ID NO: 92: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 97 :EEGAVVFPVLHG, 1.237,83->94
Second predicted epitopic region of SEQ ID NO: 92: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 98 :TKRVLESAGIA-QVPYVAIVEGD, 1.227,130->151
Third predicted epitopic region of SEQ ID NO: 92: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 99 :DSRVLVEQG, 1.163,201->209

- 5                   <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Eschericia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpxx* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

**FIGURE 81**

SEQ ID NO: 100

ATGGGTAAATATTTTGGGACTGATGGAGTCCGTGGAGAAGCTAACCTAG  
5 AACTAACACCAGAATTAGCCTTTAAACTAGGACGTTTTGGAGGCTATGTTCTTA  
GTCAACATGAAACGGAAGCGCCGAAAGTCTTTGTAGGACGTGACACACGTATT  
TCAGGGGAAATGTTGGAATCGGCCTTGGTGGCAGGTCTCCTTTCAGTAGGGATT  
CACGTATACAACTTGGTGTCTTGCAACACCAGCAGTAGCTTACTTGGTTGAA  
ACTGAAGGAGCAAGTGCCGGTGTGATGATTTCTGCTAGCCACAACCCAGCCCTT  
10 GATAACGGAATCAAGTTCTTTGGCGGTGATGGCTTCAAACCTAGATGATGAAAA  
AGAAGCAGAAATTGAAGCCTTGCTAGATGCTGAGGAAGACACTCTTCCTCGTC  
CAAGTGCAGAAGGCTTAGGAATTTTGGTAGATTATCCAGAAGGCTTGCGTAAG  
TATGAAGGATACCTTGTTTCAACTGGAACCTCCTTGTGATGGAATGAAGGTTGCC  
TTGGATACAGCTAATGGAGCAGCTTCTACCAGTGCCCGTCAAATCTTTGCAGAC  
15 CTTGGTGCCCAATTGACGGTTATCGGGGAAACACCAGACGGTCTTAACATCAAC  
CTTAATGTTGGTTCAACACATCCAGAAGCCCTTCAAGAAGTGGTCAAAGAAAG  
TGGGTCAGCTATTGGTTTGGCCTTTGATGGAGACAGTGACCGCTTGATTGCTGT  
TGATGAGAATGGTGACATCGTTGATGGTGACAAGATTATGTACATCATCGGAA  
AATACCTTTCTGAAAAAGGACAATTGGCTCAAATACAATTGTGACAACTGTTA  
20 TGTCTAACCTTGGTTTCCACAAGGCCTTGAATCGCGAAGGTATTAACAAGGCAG  
TACTGCAGTTGGTGACCGCTACGTTGTTGAAGAAATGAGAAAATCAGGCTAC  
AACCTTGGTGGTGAACAGTCTGGTCACGTTATCTTGATGGATTACAATACCACA  
GGTGATGGTCAATTATCAGCAGTTCAATTGACTAAAATCATGAAGGAAACTGG  
TAAGAGCTTATCAGAGTTGGCGGCAGAAAGTAACGATTTATCCACAAAAATTAG  
25 TTAATATCCGAGTGGAACCGTCATGAAGGAAAAGGCCATGGAAGTGCCAGCT  
ATCAAGGCCATCATCGAGAAGATGGAAGAAGAAATGGCGGGGAACGGCCGTA  
TCCTTGTTTCGTCCAAGTGGAACAGAACCCCTCTTGCGTGTTATGGCAGAAGCGC  
CTACAACAGAAGAAGTAACTACTATGTTGATACCATCACAGATGTAGTTCGTG  
CTGAAATTGGGATTGACTAA

**FIGURE 82**

SEQ ID NO: 101

MGKYFGTDGVRGEANLELTPELAFKLGRFGGYVLSQHETEAPKV FVGRDT  
5 RISGEMLESALVAGLLSVGIHVYKLGVLATPAVAYLVETEGASAGVMISASHNPAL  
DNGIKFFGGDGFKLDDEKEAEIEALLDAEEDTLPRPSAEGLGILVDYPEGLRKYEGY  
LVSTGTPLDGMKVALDTANGAASTSARQIFADLGAQLTVIGETPDGLNINLNVGST  
HPEALQEVVKESGSAIGLAFDGDSDRLIAVDENGDIVDGDKIMYIIGKYLSEKGQLA  
QNTIVTTVM SNLGFHKALNREGINKAVTAVGDRYVVEEMRKSGYNLGGEQSGHVI  
10 LMDYNTTGDGQLSAVQLTKIMKETGKSLSELAAEVTIYPQKLVNIRVENVMKEKA  
MEVPAIKAIIEKMEEEMAGNGRILVRPSGTEPLLRVMAEAPTTEE VNYVDTITDV  
VRAEIGID

**FIGURE 83**

SEQ ID NO: 102

ATGGGTAAATATTTTGGGACTGATGGAGTCCGTGGAGAAGCTAACCTAG  
5 AACTAACACCAGAATTAGCCTTTAAACTAGGACGTTTTTGGAGGCTATGTTCTTA  
GTCAACATGAAACGGAAGCGCCGAAAGTCTTTGTAGGACGTGACACACGTATT  
TCAGGGGAAATGCTGGAATCGGCCTTGGTGGCAGGTCTCCTTTCAGTAGGGATT  
CACGTATACAAACTTGGTGTCTTGCAACATCAGCAGTAGCTTACTTGGTTGAA  
ACTGAAGGAGCAAGTGCCGGTGTGATGATTTCTGCTAGCCACAACCCAGCCCTT  
10 GATAACGGAATCAAGTTCTTTGGCGGTGATGGCTTCAAACCTAGATGATGAAAA  
AGAAGCAGAAATTGAAGCCTTGCTAGATGCTGAGGAAGACACTCTTCCTCGGC  
CAAGTGCAGAAGGTTTAGGAATCTTGGTAGATTATCCAGAAGGCTTGCGTAAG  
TATGAAGGATACCTTGTCTCAACTGGAACCTCTTGATGGAATGAAGGTTGCC  
TTGGATACAGCTAATGGAGCAGCTTCTACCAGTGCCCGTCAAATCTTTGCAGAC  
15 CTTGGTGCCCAATTGACGGTTATCGGGGAAACACCAGACGGTCTTAACATCAAC  
CTTAATGTTGGTTCAACACATCCAGAAGCCCTTCAAGAAGTGGTCAAAGAAAG  
TGGGTCAGCTATTGGTTTGGCCTTTGATGGAGACAGTGACCGCTTGATTGCTGT  
TGATGAGAATGGTGACATCGTTGATGGTGACAAGATTATGTACATCATCGGAA  
AATACCTTTCTGAAAAAGGACAATTGGCTCAAAATACAATTGTGACAACCTGTTA  
20 TGTCTAACCTTGGTTTCCACAAGGCCTTGAATCGCGAAGGTATTAACAAGGCAG  
TACTGCAGTTGGTGACCGCTACGTTGTTGAAGAAATGAGAAAATCAGGCTAC  
AACCTTGGTGGTGAACAGTCTGGTCACGTTATCTTGATGGATTACAATACCACA  
GGTGATGGTCAATTATCAGCAGTTCAATTGACTAAAATCATGAAGGAAACTGG  
TAAGAGCTTATCAGAGTTGGCGGCAGAAGTAACGATTTATCCACAAAAATTAG  
25 TTAATATCCGAGTGGAACACGTCATGAAGGAAAAGGCCATGGAAGTGCCAGCT  
ATCAAGGCCATCATCGAGAAGATGGAAGAAGAAATGGCGGGGAACGGCCGTA  
TCCTTGTTTCGTCCAAGTGGAACAGAACCCCTCTTGCGTGTTATGGCAGAAGCGC  
CTACAACAGAAGAAGTAACTACTATGTTGATACCATCACAGATGTAGTTCGTG  
CTGAAATTGGGATTGACTAA

**FIGURE 84**

SEQ ID NO: 103

MGKYFGTDGVRGEANLELTPELAFKLGRFGGYVLSQHETEAPKV FVGRDT  
5 RISGEMLESALVAGLLSVGIHVYKLGVLATSAVAYLVETEGASAGVMISASHNPAL  
DNGIKFFGGDGFKLDDEKEAEIEALLDAEEDTLPRPSAEGLGILVDYPEGLRKYEGY  
LVSTGTPLDGMKVALDTANGAASTSARQIFADLGAQLTVIGETPDGLNINLNVGST  
HPEALQEVVKESGSAIGLAFDGDSDRLIAVDENGDIVDGDKIMYIIGKYLSEKGQLA  
QNTIVTTVMSNLGFHKALNREGINKAVTAVGDRYVVEEMRKSGYNLGGEQSGHVI  
10 LMDYNTTGDGQLSAVQLTKIMKETGKSLSELAAEVTIYPQKLVNIRVENVMKEKA  
MEVPAIKAIIEKMEEEMAGNGRILVRPSGTEPLLRVMAEAPTTEEVNYYVDTITDV  
VRAEIGID

**FIGURE 85**

SEQ ID NO: 104

Forward PCR Primer

5 GCGGCGGCCCATATGGGTAAATATTTGGGACTG

SEQ ID NO: 105

10

Reverse PCR Primer

GCGCGGATCCGTCAATCCCAATTCAGCAC

**FIGURE 86****TABLE 17 Properties of phosphoglucomutase/phosphomannomutase family protein from *S. pneumoniae***

TABLE 17 -- phosphoglucomutase/phosphomannomutase family protein from <i>S. pneumoniae</i> -- SEQ ID NO: 100-SEQ ID NO: 103	
Melting temperature (°C) of SEQ ID NO: 104 (forward PCR primer)	60
Restriction enzyme for SEQ ID NO: 104 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 105 (reverse PCR primer)	58
Restriction enzyme for SEQ ID NO: 105 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 100	1353
Number of amino acid residues in SEQ ID NO: 101	450
Number of different nucleic acid residues between SEQ ID NO: 100 and SEQ ID NO: 102	5
Number of different amino acid residues between SEQ ID NO: 101 and SEQ ID NO: 103	1
Calculated molecular weight of SEQ ID NO: 101 polypeptide (kDa)	48.1
Calculated pI of SEQ ID NO: 101 polypeptide	4.4
Solubility of SEQ ID NO: 103 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching 100%
Solubility of SEQ ID NO: 103 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	Approximately two thirds
Amount of purified selmet labeled polypeptide having SEQ ID NO: 103, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 3 at the C-terminus as described in EXAMPLE 6.	5.8
Amount of purified selmet labeled polypeptide having SEQ ID NO: 103 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	23.9

**FIGURE 86-B**

**TABLE 17 continued: Properties of phosphoglucomutase/phosphomannomutase family protein from *S. pneumoniae***

TABLE 17 -- phosphoglucomutase/phosphomannomutase family protein from <i>S. pneumoniae</i> -- SEQ ID NO: 100-SEQ ID NO: 103	
Z-score for the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 103, determined as described in EXAMPLE 9	4.7E-05
Number of matched peptides in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 103, determined as described in EXAMPLE 9	14
Minimum sequence coverage in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 103, determined as described in EXAMPLE 9	36
Calculated molecular weight of SEQ ID NO: 101 polypeptide (Da), determined as described in EXAMPLE 10	49717
Experimental molecular weight of SEQ ID NO: 103 polypeptide (Da), determined as described in EXAMPLE 10	49327
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. No interacting proteins were observed by using at least one of the methods described in those examples.	



FIGURE 86-C

**TABLE 17 continued: Truncation Polypeptides of phosphoglucosyltransferase/phosphomannomutase family protein from *S. pneumoniae***  
 phosphoglucosyltransferase/phosphomannomutase family protein from *S. pneumoniae* -- SEQ ID NO: 100-SEQ ID NO: 103

Start of truncated polypeptide of SEQ ID NO: 103	K3	K3	F5	G9
End of truncated polypeptide of SEQ ID NO: 103	T440	V442	G448	T440
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approximately two-thirds	Approximately two-thirds	Approaching one-third	Approaching one-third
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	Approximately two-thirds	Approximately two-thirds	Approaching one-third	No discernable expression
Amount of purified truncated polypeptide, prepared and purified as described in the Exemplification (mg/L of culture).	7.2 (1)	0.7 (1)	1.9 (2)	1.7 (3)
Amount of purified, truncated polypeptide soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	36	7	10	13
Z-score for the peptide fingerprint mapping analysis of truncated polypeptide, determined as described in EXAMPLE 9	1.60E-06	8.8E-04	3.7E-04	2.1E-04
Number of matched peptides in the peptide fingerprint mapping analysis of truncated polypeptide, determined as described in EXAMPLE 9	14	11	9	13
Minimum sequence coverage in the peptide fingerprint mapping analysis of truncated polypeptide, determined as described in EXAMPLE 9	33%	22%	21%	34%
Calculated molecular weight of truncated polypeptide (Da), determined as described in EXAMPLE 10	48814	49028	49362	48315
Experimental molecular weight of truncated polypeptide (Da), determined as described in EXAMPLE 10	48786	49038	49317	48413

- (1) The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 3 at the C-terminus as described in EXAMPLE 6.
- (2) The polypeptide so expressed and purified has the additional amino acid residues of SEQ ID NO: 2 from the removed His tag at the C-terminus as described in EXAMPLE 6.
- (3) The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.

FIGURE 86-D

TABLE 17 continued: Truncation Polypeptides of phosphoglucomutase/phosphomannomutase family protein from *S. pneumoniae*

phosphoglucomutase/phosphomannomutase family protein from <i>S. pneumoniae</i> -- SEQ ID NO: 100-SEQ ID NO: 103				
Start of truncated polypeptide of SEQ ID NO: 103	F5	F5	F5	F5
End of truncated polypeptide of SEQ ID NO: 103	V442	R444	E446	T440
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one-third	Approaching one-third	Approaching one-third	Approaching one-third
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	No discernable expression		No discernable expression	No discernable expression

phosphoglucomutase/phosphomannomutase family protein from <i>S. pneumoniae</i> -- SEQ ID NO: 100-SEQ ID NO: 103				
Start of truncated polypeptide of SEQ ID NO: 103	T7	T7	T7	T7
End of truncated polypeptide of SEQ ID NO: 103	T440	V442	R444	E446
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one-third	Approaching one-third		
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	No discernable expression		No discernable expression	No discernable expression

phosphoglucomutase/phosphomannomutase family protein from <i>S. pneumoniae</i> -- SEQ ID NO: 100-SEQ ID NO: 103				
Start of truncated polypeptide of SEQ ID NO: 103	T7	K3	G9	G9
End of truncated polypeptide of SEQ ID NO: 103	G448	R444	V442	R444
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)		No discernable expression		Approximately one-third
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	No discernable expression	No discernable expression	No discernable expression	No discernable expression

FIGURE 86-E

TABLE 17 continued: Truncation Polypeptides of phosphoglucomutase/phosphomannomutase family protein from *S. pneumoniae*

phosphoglucomutase/phosphomannomutase family protein from <i>S. pneumoniae</i> -- SEQ ID NO: 100-SEQ ID NO: 103			
Start of truncated polypeptide of SEQ ID NO: 103	G9	R11	R11
End of truncated polypeptide of SEQ ID NO: 103	E446	T440	V442
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)		Approaching one-third	Approachingly two-thirds
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	Approximately two-thirds	No discernable expression	Approaching one-third

phosphoglucomutase/phosphomannomutase family protein from <i>S. pneumoniae</i> -- SEQ ID NO: 100-SEQ ID NO: 103			
Start of truncated polypeptide of SEQ ID NO: 103	R11	R11	R11
End of truncated polypeptide of SEQ ID NO: 103	R444	E446	G448
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one-third	Approaching one-third	Approaching one-third
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	No discernable expression	No discernable expression	

FIGURE 86-F

TABLE 17 continued: Truncation Polypeptides of phosphoglucomutase/phosphomannomutase family protein from *S. pneumoniae*

PCR primers and restriction enzymes used to prepare the truncated polypeptides of TABLE 17, and the deleted amino acid residues in them, are set forth in the following tables:

Start of truncated polypeptide	K3	F5	T7	G9	R11
Residues deleted from N-terminus	MG	MGKY	MGKYFG	MGKYFGTD	MGKYFGTDGV
Nucleic acid sequence of forward PCR primer	SEQ ID NO: 106 G CGCGGGCCCA TATGAAATATT TTGGGACTGAT G	SEQ ID NO: 107 G CGCGGGCCCA TATGTTTGGG ACTGATGGAG TC	SEQ ID NO: 108 G CGCGGGCCCA TATGACTGATG GAGTCCGTG	SEQ ID NO: 109 G CGCGGGCCCA TATGGGAGTC CGTGGAGAAG	SEQ ID NO: 110 G CGCGGGCCCA TATGCGTGGA GAAGCTAACC
Restriction enzyme for forward PCR primer	NdeI	NdeI	NdeI	NdeI	NdeI

End of truncated polypeptide	T440	V442	R444	E446	G448
Residues deleted from C-terminus	DVVR AEIGID	VRAEIGID	AEIGID	IGID	ID
Nucleic acid sequence of reverse PCR primer	SEQ ID NO: 111 G CGCGGATCCT GTGATGGTATC AACATAGTAG	SEQ ID NO: 112 G CGCGGATCCT ACATCTGTGAT GGTATCAAC	SEQ ID NO: 113 G CGCGGATCCA CGAACTACAT CTGTGATGG	SEQ ID NO: 114 G CGCGGATCCTT CAGCACGAAC TACATCTG	SEQ ID NO: 115 G CGCGGATCCC CCAATTTCAGC ACGAACCTAC
Restriction enzyme for reverse PCR primer	BamHI	BamHI	BamHI	BamHI	BamHI

A blank in any of the parts of TABLE 17 indicates that the experiment was not completed.

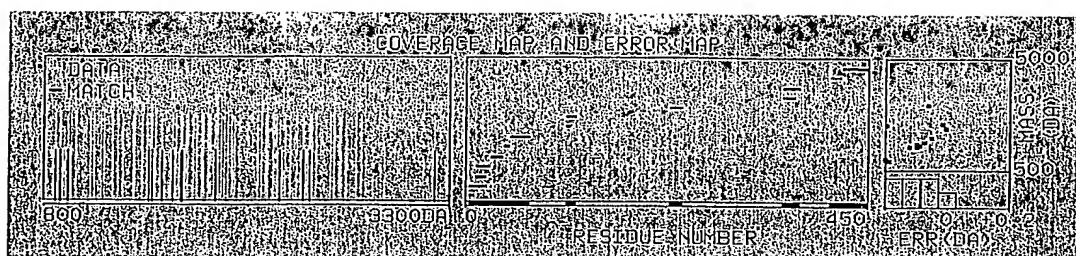
## FIGURE 87

**TABLE 18 Bioinformatic Analyses of phosphoglucomutase/phosphomannomutase family protein from *S. pneumoniae***

TABLE 18 -- phosphoglucomutase/phosphomannomutase family protein from <i>S. pneumoniae</i> -- SEQ ID NO: 100-SEQ ID NO: 103	
COG Category	Carbohydrate transport and metabolism
COG ID Number	COG1109
Is SEQ ID NO: 101 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 101	None
Source organism for closest PDB protein to SEQ ID NO: 101	N/A
e-value for closest PDB Protein to SEQ ID NO: 101	N/A
% Identity between SEQ ID NO: 101 and the closest protein from PDB	N/A
% Positives between SEQ ID NO: 101 and the closest protein from PDB	N/A
Number of Protein Hits in the VGDB to SEQ ID NO: 101	13
Number of Microorganisms having VGDB Hits to SEQ ID NO: 101	10
Microorganisms having VGDB Hits to SEQ ID NO: 101 <sup>1</sup>	[spne][bsub][saur][paer][ctra][ecoli][nmen][hinf][hpyl][efae]
First predicted epitopic region of SEQ ID NO: 101: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 116 :ESALVAGLLSV GIH-VYKLGVLATPAVAYLVET, 1.196,58->89
Second predicted epitopic region of SEQ ID NO: 101: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 117 :LSAVQLTK, 1.141,343->350
Third predicted epitopic region of SEQ ID NO: 101: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 118 :LSELAAEVTIYP Q-KLVNIRVEN, 1.136,359->380

- 5 <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Eschericia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpax* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

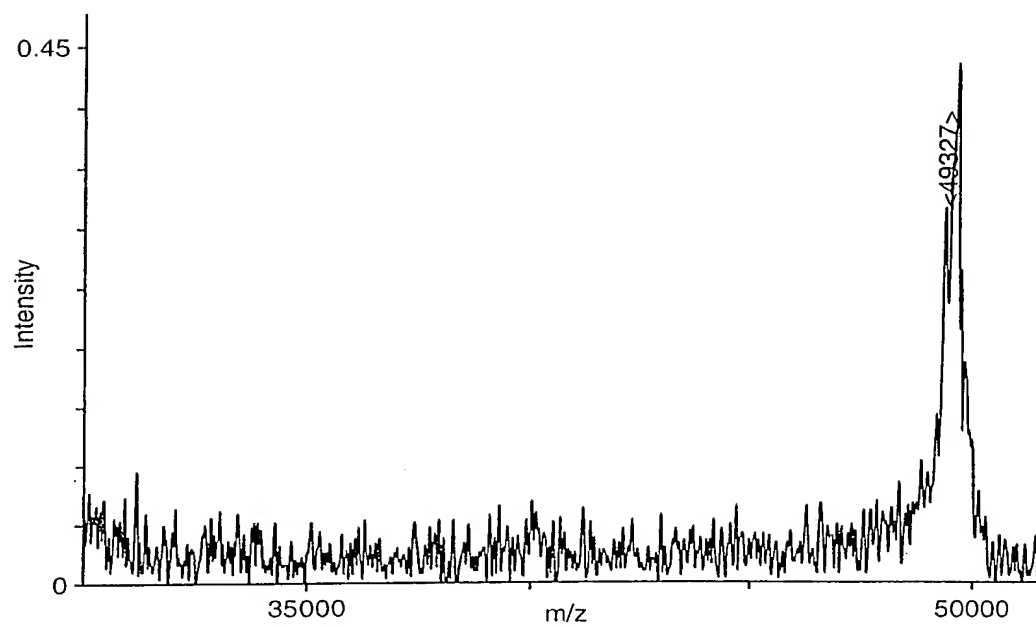
FIGURE 88



Note: click on the ☐ symbol to change column format.

Measured Mass (k)	Avg/ Mono	Computed Mass	Error: <input type="checkbox"/> (Da)	<input type="checkbox"/> Residues Start	Missed To	Cut	Peptide sequence
873.246	M	873.402	-0.156	112	119	0	FFGGDGFK
913.261	M	913.429	-0.168	4	11	0	YFGTDGVR
948.350	M	948.514	-0.164	44	51	1	VFVGRDTR
1449.768	M	1449.866	-0.098	409	421	0	ILVRPSGTEPLLR
1473.594	M	1473.677	-0.083	112	124	1	FFGGDGFKLDDEK
1530.719	M	1530.792	-0.073	12	25	0	GEANLELTPELAFK
1595.624	M	1595.706	-0.082	230	245	0	ESGSAIGLAFDGDSDR
1647.807	M	1647.871	-0.064	358	372	0	SLSELAAEVTIYPQK
1661.747	M	1661.804	-0.057	29	43	0	FGGYVLSQHETAPK
1856.960	M	1856.999	-0.039	12	28	1	GEANLELTPELAFKLGR
2243.250	M	2243.251	-0.001	358	377	1	SLSELAAEVTIYPQKLVNIR
2385.137	M	2385.297	-0.159	52	74	0	ISGENLESALVAGLLSVGIHVYK
2426.200	M	2426.210	-0.010	4	25	1	YFGTDGVRGEANLELTPELAFK
3212.493	M	3212.542	-0.049	422	450	1	VMAEAPTTEEVNYYVDTITDVVRAEIGID

FIGURE 89



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FIGURE 90

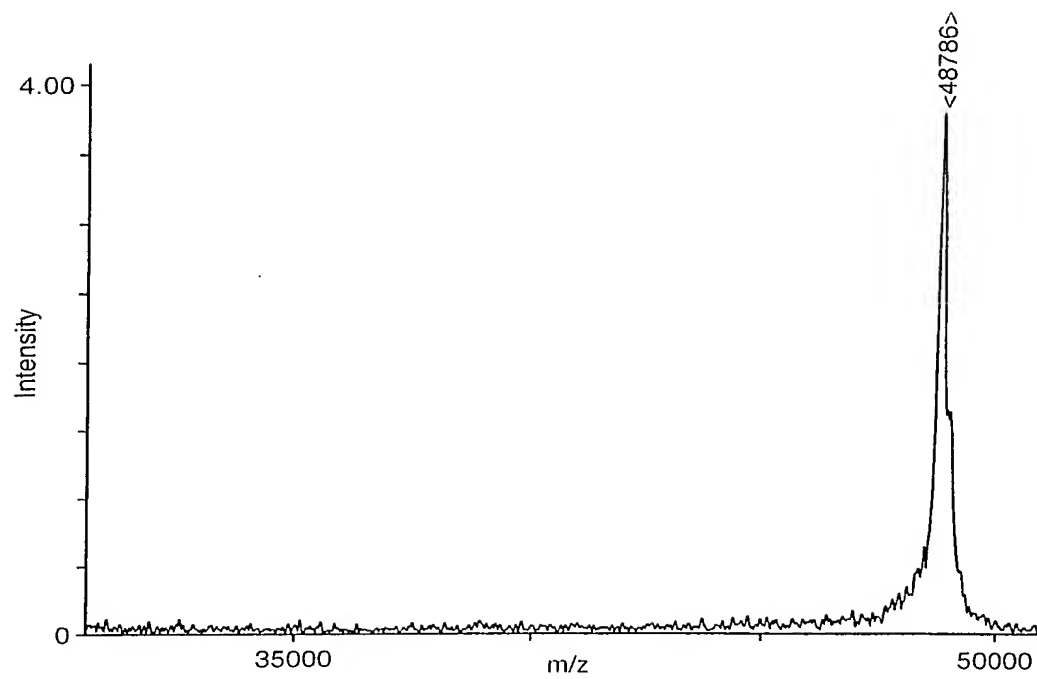
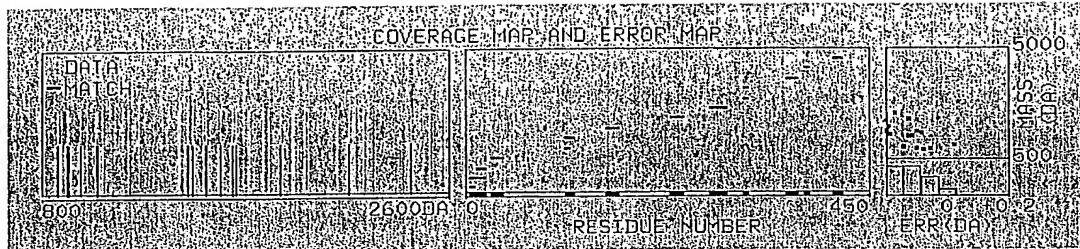




FIGURE 91



Note: click on the ☐ symbol to change column format.

Measured	Avg/	Computed	Error	<input checked="" type="checkbox"/> Residues	Missed	
Mass(M)	Mono	Mass	(Da)	Start	To	Cut Peptide sequence
873.332	M	873.402	-0.069	112	119	0 FFGGDGPK
913.342	M	913.429	-0.087	4	11	0 YFGTDGVR
924.386	M	924.437	-0.051	310	316	0 YVVEEMR
975.372	M	975.505	-0.133	378	385	1 VENVMKEK
1052.553	M	1052.532	0.021	310	317	1 YVVEEMRK
1449.763	M	1449.866	-0.103	409	421	0 ILVRPSGTEPLLR
1473.559	M	1473.677	-0.118	112	124	1 FFGGDGFKLDDEK
1530.620	M	1530.792	-0.172	12	25	0 GEANLELTPELAFK
1595.630	M	1595.706	-0.076	230	245	0 ESGSAIGLAFDGDSDR
1647.686	M	1647.871	-0.185	358	372	0 SLSELAAEVTIYPQK
1661.712	M	1661.804	-0.092	29	43	0 FGGYVLSQHETEAPK
1857.741	M	1857.917	-0.176	159	175	1 KYEGYLVSTGTPLDGMK
2158.004	M	2158.119	-0.115	273	292	0 QQLAQTIVTTVMSNLGFHK
2426.054	M	2426.210	-0.156	4	25	1 YFGTDGVRGEANLELTPELAFK

FIGURE 92

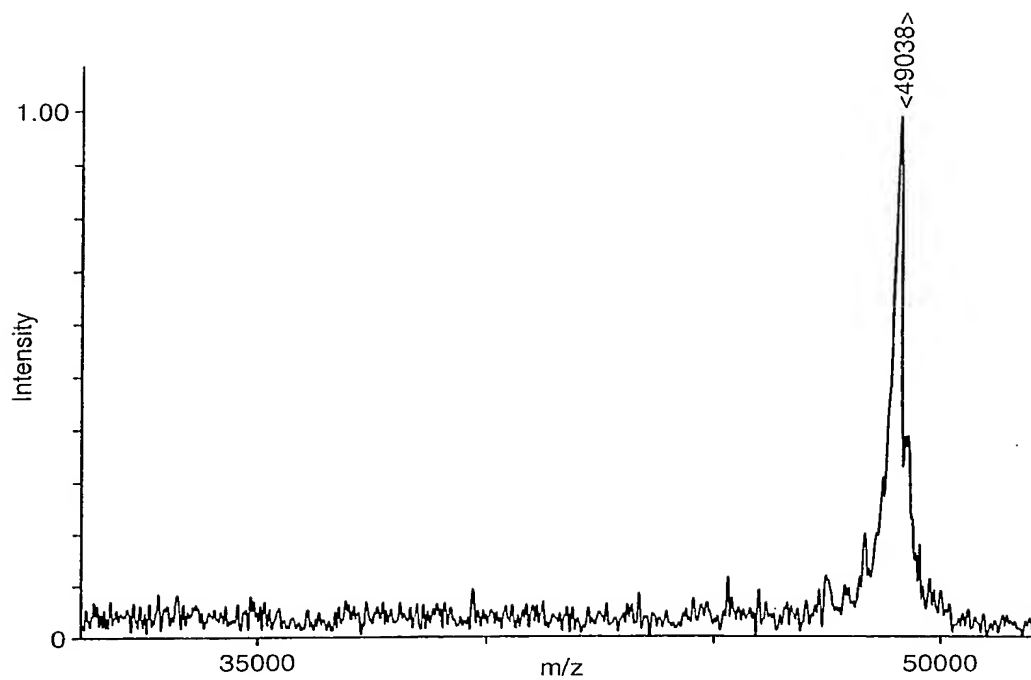
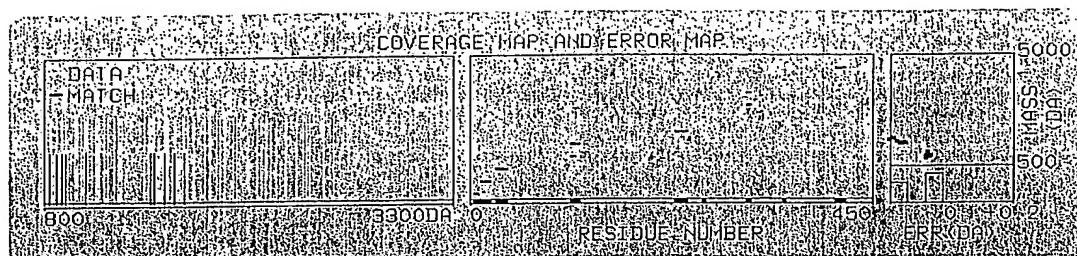


FIGURE 93



Note: click on the ☐ symbol to change column format.

Measured Mass (M)	Avg/ Mono	Computed Mass	Error (Da)	<input type="checkbox"/> Residues	Missed	Peptide sequence
805.357	M	805.436	-0.079	351	357	1 IMKETGK
836.402	M	836.482	-0.080	261	267	0 IMYIIGK
873.331	M	873.402	-0.071	112	119	0 FFGDGPFK
913.364	M	913.429	-0.065	4	11	0 YFGTDGVR
924.363	M	924.437	-0.074	310	316	0 YVVEEMR
1052.458	M	1052.532	-0.073	310	317	1 YVVEEMRK
1449.707	M	1449.866	-0.159	409	421	0 ILVRPSGTEPLLR
1473.532	M	1473.677	-0.145	112	124	1 FFGDGFKLDDEK
1530.619	M	1530.792	-0.173	12	25	0 GEANLELTPELAFK
1595.509	M	1595.706	-0.197	230	245	0 ESGSAIGLAFDGSDR
1661.621	M	1661.804	-0.183	29	43	0 FGGYVLSQHETEAPK

FIGURE 94

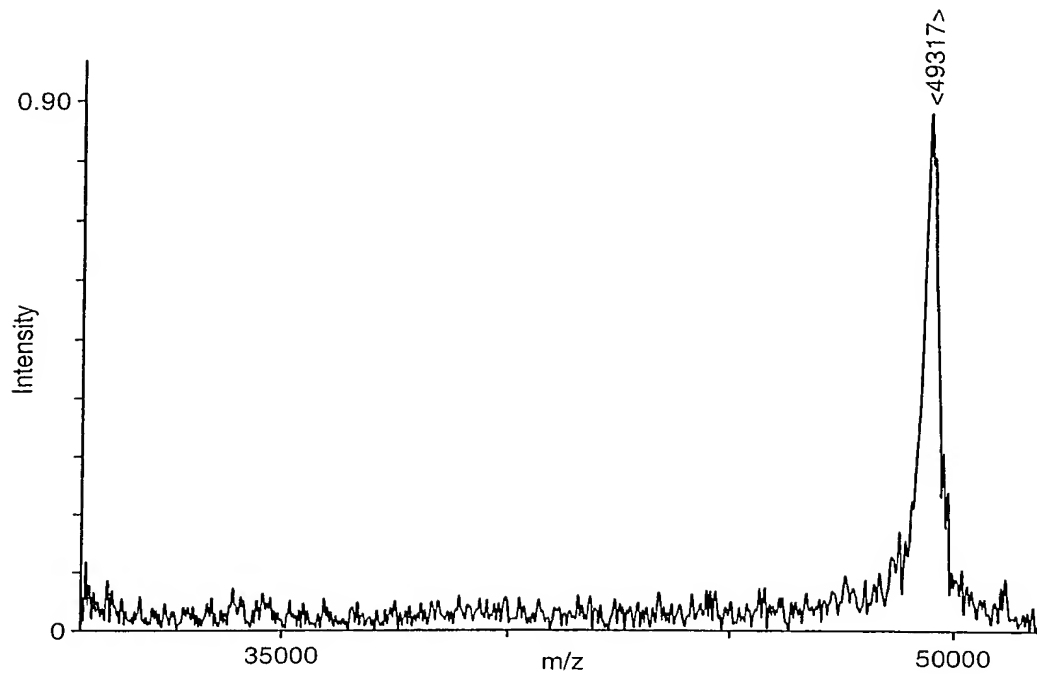


FIGURE 95



Note: click on the ☐ symbol to change column format.

Measured Mass (M)	Avg/ Mono	Computed Mass	Error (Da)	<input checked="" type="checkbox"/> Residues	Missed	Cut	Peptide sequence
873.309	M	873.402	-0.092	112	119	0	FFGGDGFK
924.318	M	924.437	-0.119	310	316	0	YVVEEMR
1052.385	M	1052.532	-0.147	310	317	1	YVVEEMRK
1403.509	M	1403.700	-0.191	176	190	0	VALDTANGAASTSAR
1449.707	M	1449.866	-0.159	409	421	0	ILVRPSGTEPLL
1473.532	M	1473.677	-0.145	112	124	1	FFGGDGFKLDDEK
1530.620	M	1530.792	-0.173	12	25	0	GEANLELTPELAFK
1595.538	M	1595.706	-0.167	230	245	0	ESGSAIGLAFDGDSDR
1661.653	M	1661.804	-0.151	29	43	0	FGYVLSQHETEAPK

FIGURE 96

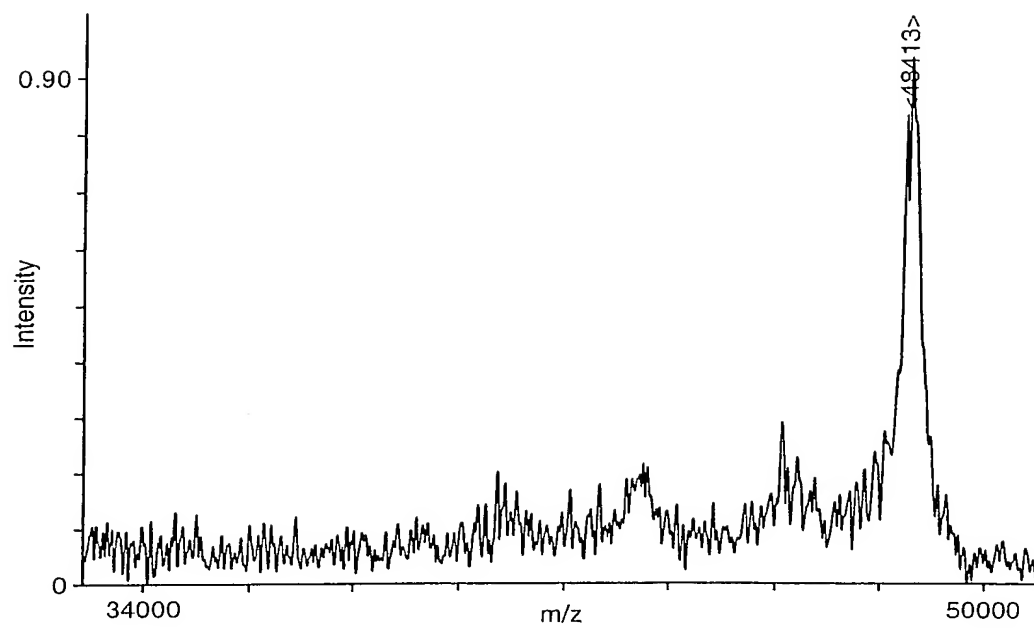


FIGURE 97



Note: click on the ☐ symbol to change column format.

Measured	Avg/	Computed	Error	<div><div></div>Residues</div>	Missed		
Mass (M)	Mono	Mass	<div><div></div>(Da)</div>	Start	To	Cut	Peptide sequence
873.422	M	873.402	0.021	112	119	0	FFGGDGFK
924.457	M	924.437	0.020	310	316	0	YVVEEMR
1052.510	M	1052.532	-0.021	310	317	1	YVVEEMRK
1122.402	M	1122.443	-0.040	399	408	0	MEEEMAGNR
1449.853	M	1449.866	-0.013	409	421	0	ILVRPSGTEPLLR
1473.709	M	1473.677	0.032	112	124	1	FFGGDGFKLDDEK
1530.799	M	1530.792	0.007	12	25	0	GEANLELTPELAFK
1595.692	M	1595.706	-0.014	230	245	0	ESGSAIGLAFDGDSDR
1647.869	M	1647.871	-0.002	358	372	0	SLSELAAEVTIYPQK
1661.809	M	1661.804	0.005	29	43	0	FGGYVLSQHETAPK
1693.801	M	1693.845	-0.044	302	316	1	AVTAVGDRYVVEEMR
1857.907	M	1857.917	-0.010	159	175	1	KYEGYLVSTGTPLDGHK
2390.233	M	2390.239	-0.006	246	267	1	LIAVDENGDIVDGDKIMYIIGK

**FIGURE 98**

SEQ ID NO: 119

ATGAAAGTAATAGATCAATTTAAAAATAAGAAAGTCCTTGTTTTAGGTT  
5 TGGCCAAGTCTGGTGAATCTGCAGCTCGTTTGTGGACAAGCTAGGTGCCATTG  
TGACAGTAAATGATGGGAAACCTTTCGAGGACAATCCAGCTGCCCAAAGTTTG  
CTGGAAGAAGGGATCAAGGTCATTACAGGTGGCCATCCTTTGGAACCTTTGGAT  
GAAGAGTTTGCCCTTATGGTGAAAAATCCAGGTATCCCCTACAACAATCCCATG  
ATTGAAAAGGCTTTGGCCAAGGGAATTCCAGTCTTGACTGAGGTGGAATTGGCT  
10 TATTTGATTTT CAGAAGCACCGATTATTGGTATCACAGGATCGAACGGTAAGACA  
ACCACAACGACTATGATTGGGGAAGTTTTGACTGCTGCTGGCCAACATGGTCTT  
TTATCAGGGAATATCGGCTATCCAGCTAGTCAGGTTGCTCAAATAGCATCAGAT  
AAGGACACGCTTGTTATGGAACCTTCTTCTTTCCAACCTCATGGGTGTTCAAGAA  
TTCCATCCAGAGATTGCGGTTATTACCAACCTCATGCCAACTCATATCGACTAC  
15 CATGGGTCAATTTTCGGAATATGTAGCAGCCAAGTGGAATATCCAGAACAAGAT  
GACAGCAGCTGATTTCCCTTGTCTTGAACCTTAATCAAGACTTGGCAAAAGACTT  
GACTTCCAAGACAGAAGCCACTGTTGTACCATTTTCAACACTTGAAAAGGTTGA  
TGGAGCTTATCTGGAAGATGGTCAACTCTACTTCCGTGGTGAAGTAGTCATGGC  
AGCGAATGAAATCGGTGTTCCAGGTAGCCACAATGTGGAAAATGCCCTTGCGA  
20 CTATTGCTGTAGCCAAGCTTCGTGATGTGGACAATCAAACCATCAAGGAACTC  
TTTCAGCCTTCGGTGGTGTCAAACACCGTCTCCAGTTTGTGGATGACATCAAGG  
GTGTTAAATTCTATAACGACAGTAAATCAACTAATATCTTGGCTACTCAAAAAG  
CCTTGTCAGGATTTGACAACAGCAAGGTCGTCTTGATTGCAGGTGGTTTGGACC  
GTGGCAATGAGTTTGACGAATTGGTGCCAGACATTACTGGACTCAAGAAGATG  
25 GTCATCCTGGGTCAATCTGCAGAACGTGTCAAACGGGCAGCAGACAAGGCTGG  
TGTCGCTTATGTGGAGGCGACAGATATTGCAGATGCGACCCGCAAGGCCTATG  
AGCTTGCGACTCAAGGAGATGTGGTTCTTCTTAGTCCTGCCAATGCTAGCTGGG  
ATATGTATGCTAACTTTGAAGTACGTGGCGACCTCTTTATCGACACAGTAGCGG  
AGTTAAAAGAATAA



**FIGURE 99**

SEQ ID NO: 120

MKVIDQFKNKKVLVLGLAKSGESAARLLDKLGAIVTVNDGKPFEDNPAAQ  
5 SLLEEGIKVITGGHPLELLDEEFALMVKNPGIPYNNPMIEKALAKGIPVLTEVELAYL  
ISEAPIIGITGSNGKTTTTTMMIGEVLTAAGQHGLLSGNIGYPASQVAQIASDKDTLVM  
ELSSFQLMGVQEFHPEIAVITNLMPTHIDYHGSFSEYVAAKWNIQNKMTAADFLVL  
NFNQDLAKDLTSKTEATVVPFSTLEKVDGAYLEDGQLYFRGEVVMAANEIGVPGS  
HNVENALATI AVAKLRDVDNQTIKETLSAFGGVKHRLQFVDDIKGVKFYNDKST  
10 NILATQKALSGFDNSKVVLIAGGLDRGNEFDELVPDITGLKKMVILGQSAERVKRA  
ADKAGVAYVEATDIADATRKAYELATQGDVLLSPANASWDMYANFEVRGDLFI  
DTV AELKE

**FIGURE 100**

SEQ ID NO: 121

ATGAAAGTAATAGATCAATTTAAAAATAAGAAAGTCCTTGTTTTAGGTT  
5 TGGCCAAGTCTGGTGAATCTGCAGCTCGTTTGTGGACAAGCTAGGTGCCATTG  
TGACAGTAAATGATGGGAAGCCTTTCGAGGACAATCCAGCTGCCCAAAGTTTG  
CTGGAAGAAGGGATCAAGGTCATTACAGGTGGCCATCCTTTGGAACCTTTGGAT  
GAAGAGTTTGGCCTTATGGTGAAAAATCCAGGTATCCCCTACAACAATCCCATG  
ATTGAAAAGGCTTTGGCCAAGGGAATTCCAGTCTTGACTGAGGTGGAATTGGCT  
10 TATTTGATTTTCTAGAAGCACCGATTATTGGTATCACAGGATCGAACGGTAAGACA  
ACCACAACGACTATGATTGGGGAAGTTTTGACTGCTGCTGGGCAACATGGTCTT  
TTATCAGGGAATATCGGCTATCCTGCCAGTCAGGTTGCTCAAATAGCATCAGAT  
AAGGATACGCTTGTTATGGAACCTTCTTCTTTCCAACCTCATGGGTGTTCAAGAA  
TTCCATCCAGAGATTGCGGTTATTACCAACCTCATGCCAACTCATATCGACTAC  
15 CATGGGTCATTTTCGGAATATGTAGCAGCCAAGTGGAATATCCAGAACAAGAT  
GACAGCAGCTGATTTCTTGTCTTGAACCTTAAATCAAGACTTGGCAAAAGACTT  
GACTTCCAAGACAGAAGCCACTGTTGTACCATTTTCAACACTTGAAAAGGTTGA  
TGGAGCTTATCTAGAAGATGGTCAACTCTACTTCCGTGGTGAAGTAGTCATGGC  
AGCGAATGAAATCGGTGTTCCAGGTAGCCACAATGTGGAAAATGCCCTTGCGA  
20 CTATTGCTGTAGCCAAGCTTCGTGGTGTGGACAATCAAACCATCAAGGAACTC  
TTTCAGCCTTCGGTGGTGTCAAACACCGTCTCCAGTTTGTGGATGACATCAAGG  
GTGTTAAATTCTATAACGACAGTAAATCAACTAATATCTTGGCTACTCAAAAAG  
CCTTGTCAGGATTTGACAACAGCAAGGTCGTCTTGATTGCAGGTGGTTTGGACC  
GTGGCAATGAGTTTGACGAATTGGTGCCAGATATTACTGGACTCAAGAAGATG  
25 GTCATCCTGGGTCAATCTGCAGAACGTGTCAAACGGGCAGCAGACAAGGCTGG  
TGTCGCTTATGTGGAGGCGACAGATATTGCAGATGCGACCCGCAAGGCATATG  
AGCTTGCGACTCAAGGAGATGTGGTTCTTCTTAGTCCTGCCAATGCCAGCTGGG  
ATATGTATGCTAACTTTGAAGTACGTGGCGACCTCTTTATCGACACAGTAGCGG  
AGTTAAAAGAATAA

**FIGURE 101**

SEQ ID NO: 122

MKVIDQFKNKKVLVLGLAKSGESAARLLDKLGAIVTVNDGKPFEDNPAAQ  
5 SLLEEGIKVITGGHPLELLDEEFALMVKNPGIPYNNPMIEKALAKGIPVLTEVELAYL  
ISEAPIIGITGSNGKTTTTTMMIGEVLTAAGQHGLLSGNIGYPASQVAQIASDKDTLVM  
ELSSFQLMGVQEFHPEIAVITNLMPTHIDYHGSFSEYVAAKWNIQNKMTAADFLVL  
NFNQDLAKDLTSKTEATVVPFSTLEKVDGAYLEDGQLYFRGEVVMAANEIGVPGS  
HNVENALATIATAKLRGVDNQTIKETLSAFGGVKHRLQFVDDIKGVKFYNDSKST  
10 NILATQKALSGFDNSKVVLIAAGGLDRGNEFDELVPDITGLKKMVILGQSAERVKRA  
ADKAGVAYVEATDIADATRKAYELATQGDVVLLSPANASWDMYANFEVRGDLFI  
DTVAELKE

**FIGURE 102**

SEQ ID NO: 123

Forward PCR Primer

5 GCGGCGGCCCATATGAAAGTAATAGATCAATTAAAAATAAG

SEQ ID NO: 124

10

Reverse PCR Primer

GCGCGGATCCTTCTTTAACTCCGCTACTGTG

**FIGURE 103****TABLE 19 Properties of UDP-N-acetylmuramoylalanine-D-glutamate ligase from *S. pneumoniae***

TABLE 19 -- UDP-N-acetylmuramoylalanine-D-glutamate ligase from <i>S. pneumoniae</i> -- SEQ ID NO: 119-SEQ ID NO: 122	
Melting temperature (°C) of SEQ ID NO: 123 (forward PCR primer)	70
Restriction enzyme for SEQ ID NO: 123 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 124 (reverse PCR primer)	62
Restriction enzyme for SEQ ID NO: 124 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 119	1353
Number of amino acid residues in SEQ ID NO: 120	450
Number of different nucleic acid residues between SEQ ID NO: 119 and SEQ ID NO: 121	10
Number of different amino acid residues between SEQ ID NO: 120 and SEQ ID NO: 122	1
Calculated molecular weight of SEQ ID NO: 120 polypeptide (kDa)	48.5
Calculated pI of SEQ ID NO: 120 polypeptide	4.6
Solubility of SEQ ID NO: 122 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approximately two thirds
Solubility of SEQ ID NO: 122 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	Approximately two thirds
Amount of purified selmet labeled polypeptide having SEQ ID NO: 122, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	3.6
Amount of purified selmet labeled polypeptide having SEQ ID NO: 122 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	13.4

**FIGURE 103-B**

5 **TABLE 19 continued:** Properties of UDP-N-acetylmuramoylalanine-D-glutamate ligase from *S. pneumoniae*

TABLE 19 -- UDP-N-acetylmuramoylalanine-D-glutamate ligase from <i>S. pneumoniae</i> -- SEQ ID NO: 119-SEQ ID NO: 122	
Z-score for the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 122, determined as described in EXAMPLE 9	5.3E-05
Number of matched peptides in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 122, determined as described in EXAMPLE 9	17
Minimum sequence coverage in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 122, determined as described in EXAMPLE 9	40
Calculated molecular weight of SEQ ID NO: 120 polypeptide (Da), determined as described in EXAMPLE 10	50510
Experimental molecular weight of SEQ ID NO: 122 polypeptide (Da), determined as described in EXAMPLE 10	50687
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. No interacting proteins were observed by using at least one of the methods described in those examples.	
Crystals of a selenomethionine-substituted polypeptide having the sequence of SEQ ID NO: 122, prepared and purified as described above and having a His tag, are obtained using the following conditions: 20% PEG 8000, sodium cacodylate pH 6.5, 0.2M calcium acetate. The crystals were prepared using the following method: 20°C, sitting drop, 13.4 mg polypeptide per ml of solution.	

FIGURE 103-C

TABLE 19 continued: Truncation Polypeptides of UDP-N-acetylmuramoylalanine-D-glutamate ligase from *S. pneumoniae*

UDP-N-acetylmuramoylalanine-D-glutamate ligase from <i>S. pneumoniae</i> -- SEQ ID NO: 119-SEQ ID NO: 122					
Start of truncated polypeptide of SEQ ID NO: 122	D5	D5	K8	V3	
End of truncated polypeptide of SEQ ID NO: 122	I442	A446	K449	L440	
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one-third	Approaching one-third	Approaching 100%	Approaching one-third	
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	Approximately two-thirds	Approximately two-thirds	No discernable expression		
Amount of purified truncated polypeptide, prepared and purified as described in the Exemplification (mg/L of culture).			6.5 (1)		
Amount of purified, truncated polypeptide soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)			24.0		
Z-score for the peptide fingerprint mapping analysis of truncated polypeptide, determined as described in EXAMPLE 9			4.6E-08		
Number of matched peptides in the peptide fingerprint mapping analysis of truncated polypeptide, determined as described in EXAMPLE 9			23		
Minimum sequence coverage in the peptide fingerprint mapping analysis of truncated polypeptide, determined as described in EXAMPLE 9			52%		
Calculated molecular weight of truncated polypeptide (Da), determined as described in EXAMPLE 10			49650		
Experimental molecular weight of truncated polypeptide (Da), determined as described in EXAMPLE 10			49769		

(1) The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.

FIGURE 103-D

TABLE 19 continued: Truncation Polypeptides of UDP-N-acetylmuramoylalanine-D-glutamate ligase from *S. pneumoniae*

UDP-N-acetylmuramoylalanine-D-glutamate ligase from <i>S. pneumoniae</i> -- SEQ ID NO: 119-SEQ ID NO: 122					
Start of truncated polypeptide of SEQ ID NO: 122	V3	V3	V3	D5	
End of truncated polypeptide of SEQ ID NO: 122	I442	T444	A446	L440	
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one-third	Approaching one-third	Approaching one-third	Approaching one-third	
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)			No discernable expression		

UDP-N-acetylmuramoylalanine-D-glutamate ligase from <i>S. pneumoniae</i> -- SEQ ID NO: 119-SEQ ID NO: 122					
Start of truncated polypeptide of SEQ ID NO: 122	N9	N9	K11	K11	
End of truncated polypeptide of SEQ ID NO: 122	I442	A446	L440	I442	
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)		Approaching one-third	Less than one-third	Less than one-third	
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	No discernable expression			No discernable expression	

UDP-N-acetylmuramoylalanine-D-glutamate ligase from <i>S. pneumoniae</i> -- SEQ ID NO: 119-SEQ ID NO: 122					
Start of truncated polypeptide of SEQ ID NO: 122	K11	K11	K8	V14	
End of truncated polypeptide of SEQ ID NO: 122	T444	A446	I442	I442	
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Less than one-third	Less than one-third	No discernable expression	Approaching one-third	



FIGURE 103-E

TABLE 19 continued: Truncation Polypeptides of UDP-N-acetylmuramoylalanine-D-glutamate ligase from *S. pneumoniae*

PCR primers and restriction enzymes used to prepare the truncated polypeptides of TABLE 19, and the deleted amino acid residues in them, are set forth in the following tables:

Start of truncated polypeptide	K11	K8	V14
Residues deleted from N-terminus	MKVIDQFKNK	MKVIDQF	MKVIDQFKNKKVL
Nucleic acid sequence of forward PCR primer	SEQ ID NO: 125 GCGGCG GCCCATATGAAAGTCCT TGTTTATAGTTTG	SEQ ID NO: 126 GCGGCG GCCCATATGAAAAATA AGAAAGTCCTTGTTTA G	SEQ ID NO: 127 GCGGCG GCCCATATGGTTTAGG TTTGGCCAAG
Restriction enzyme for forward PCR primer	NdeI	NdeI	NdeI

Start of truncated polypeptide	V3	D5	N9
Residues deleted from N-terminus	MK	MKVI	MKVIDQFK
Nucleic acid sequence of forward PCR primer	SEQ ID NO: 128 GCGGCG GCCCATATGGTAATAG ATCAATTAAAAATAA G	SEQ ID NO: 129 GCGGCG GCCCATATGGATCAATT TAAAAATAAGAAAG	SEQ ID NO: 130 GCGGCG GCCCATATGAATAAGA AAGTCCTTGTTTAG
Restriction enzyme for forward PCR primer	NdeI	NdeI	NdeI

End of truncated polypeptide	L440	I442	T444	A446	K449
Residues deleted from C-terminus	FIDTVaelKE	DTVaelKE	VAELKE	ELKE	E
Nucleic acid sequence of reverse PCR primer	SEQ ID NO: 131 G CGCGGATCCG AGGTCGCCAC GTACTTC	SEQ ID NO: 132 G CGCGGATCCG ATAAGAGGT CGCCACGTAC	SEQ ID NO: 133 G CGCGGATCCT GTGTCGATAA AGAGGTCGC	SEQ ID NO: 134 G CGCGGATCCC GCTACTGTGTC GATAAAGAG	SEQ ID NO: 135 G CGCGGATCCTT TTAACTCCGCT ACTGTGTC
Restriction enzyme for reverse PCR primer	BamHI	BamHI	BamHI	BamHI	BamHI

A blank in any of the parts of TABLE 19 indicates that the experiment was not completed.

## FIGURE 104

**TABLE 20 Bioinformatic Analyses of UDP-N-acetylmuramoylalanine-D-glutamate ligase from *S. pneumoniae***

TABLE 20 -- UDP-N-acetylmuramoylalanine-D-glutamate ligase from <i>S. pneumoniae</i> -- SEQ ID NO: 119-SEQ ID NO: 122	
COG Category	Cell envelope biogenesis, outer membrane
COG ID Number	COG0771
Is SEQ ID NO: 120 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 120	UDP-N-Acetylmuramoyl-L-Alanine: D-Glutamate (1eeh)
Source organism for closest PDB protein to SEQ ID NO: 120	<i>Escherichia coli</i>
e-value for closest PDB Protein to SEQ ID NO: 120	5E-45
% Identity between SEQ ID NO: 120 and the closest protein from PDB	31
% Positives between SEQ ID NO: 120 and the closest protein from PDB	48
Number of Protein Hits in the VGDB to SEQ ID NO: 120	10
Number of Microorganisms having VGDB Hits to SEQ ID NO: 120	10
Microorganisms having VGDB Hits to SEQ ID NO: 120 <sup>1</sup>	[spne][efae][bsub][saur][hinf][nmen][paer][rpxx][bbur][ctra]
First predicted epitopic region of SEQ ID NO: 120: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 136 :NKKVLVLGLA, 1.208,9->18
Second predicted epitopic region of SEQ ID NO: 120: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 137 :QGDVVLLSPA, 1.201,415->424
Third predicted epitopic region of SEQ ID NO: 120: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 138 :SKVVLIAG, 1.168,347->354

- 5                   <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Escherichia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlamydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpxx* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

FIGURE 105



Note: click on the ☐ symbol to change column format.

Measured	Avg/	Computed	Error	<input type="checkbox"/> Residues	Missed	
Mass (M)	Mono	Mass	(Da)	Start	To	Cut Peptide sequence
811.552	M	811.553	-0.001	12	19	0 VLVGLAK
939.559	M	939.648	-0.089	11	19	1 KVLVLGLAK
976.456	M	976.522	-0.067	314	321	0 LQFVDDIK
1007.465	M	1007.547	-0.081	1	8	1 MKVIDQFK
1007.465	M	1007.528	-0.063	302	311	0 ETLSAFGGVK
1011.562	M	1011.607	-0.045	349	358	0 VVLIAGGLDR
1102.520	M	1102.580	-0.060	375	384	0 MVILGQSAER
1269.626	M	1269.682	-0.056	312	321	1 HRLQFVDDIK
1319.640	M	1319.697	-0.057	438	449	0 GDLFIDTVAELEK
1420.697	M	1420.744	-0.048	236	248	0 TEATVVPFSTLEK
1485.691	M	1485.728	-0.037	79	91	0 NPGIPYNNPHIEK
1621.787	M	1621.794	-0.007	392	407	0 AGVAYVEATDIADATR
1644.772	M	1644.778	-0.006	249	262	0 VDGAYLEDGQLYFR
1749.891	M	1749.889	0.002	392	408	1 AGVAYVEATDIADATRK
1773.899	M	1773.914	-0.015	359	374	1 GNEFDLVDPDITGLKK
2210.145	M	2210.164	-0.020	59	78	0 VITGGHPELLDEEFALMVK
3047.412	M	3047.511	-0.100	236	262	1 TEATVVPFSTLEKVDGAYLEDGQLYFR
3229.453	M	3229.538	-0.085	409	437	0 AYLATQGDVVLLSPANASWDMYANFEVR

FIGURE 106

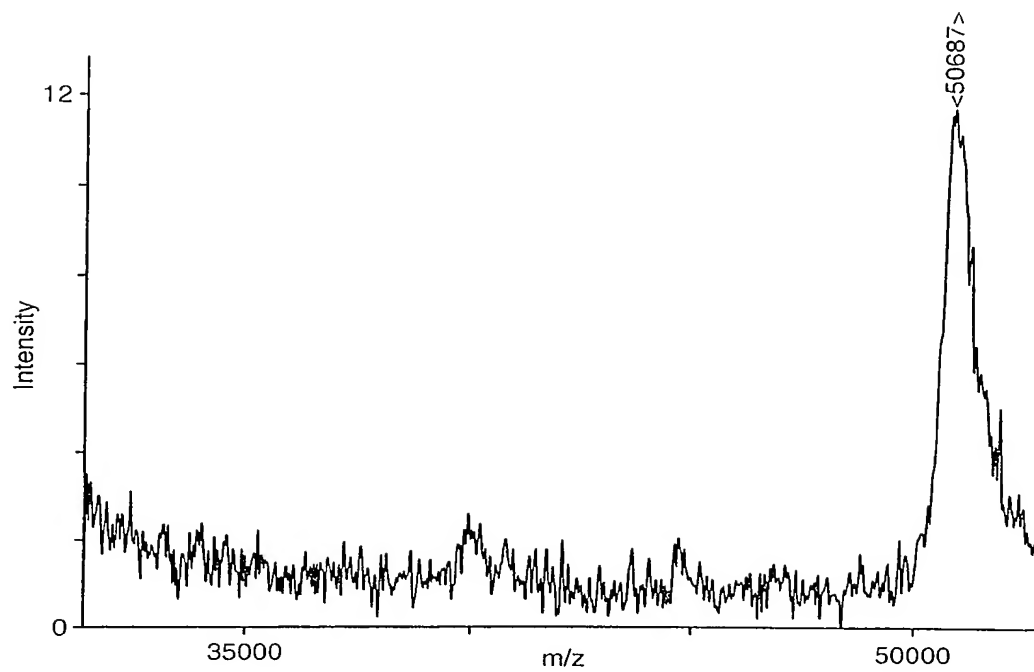


FIGURE 107

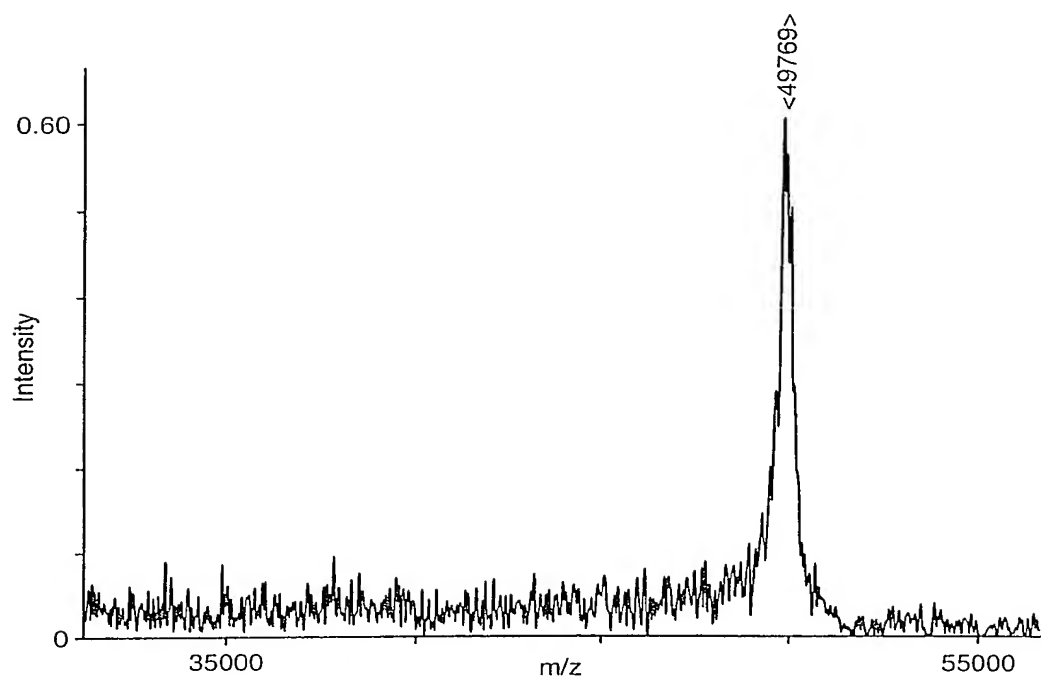
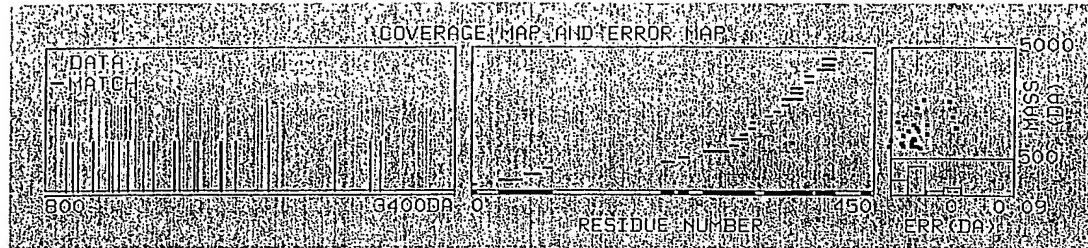


FIGURE 108



Note: click on the ☐ symbol to change column format.

Measured Mass (M)	Avg/ Mono	Computed Mass	Error (Da)	Residues Start	Residues To	Missed Cut	Peptide sequence
937.406	M	937.450	-0.044	340	348	0	ALSGFDNSK
974.489	M	974.539	-0.050	331	339	0	STHILATQK
976.474	M	976.522	-0.048	314	321	0	LQFVDDIK
1007.461	M	1007.547	-0.086	1	8	1	HKVIDQPK
1007.461	M	1007.528	-0.067	302	311	0	ETLSAFGGVK
1011.552	M	1011.607	-0.055	349	358	0	VVLIAGGLDR
1102.510	M	1102.580	-0.070	375	384	0	MVILGQSAER
1200.590	M	1200.646	-0.055	292	301	1	LRDVDNQTIK
1230.591	M	1230.675	-0.084	374	384	1	KMVLGQSAER
1269.632	M	1269.682	-0.051	312	321	1	HRLQFVDDIK
1319.642	M	1319.697	-0.054	438	449	0	GDLFIDTVAEIK
1420.677	M	1420.744	-0.067	236	248	0	TEATVVPFSTLEK
1485.693	M	1485.728	-0.034	79	91	0	NPGIPYNNPMIEK
1621.747	M	1621.794	-0.047	392	407	0	AGVAYVEATDIADTR
1645.758	M	1645.819	-0.061	359	373	0	GNEFDELVPDITGLK
1749.839	M	1749.889	-0.050	392	408	1	AGVAYVEATDIADTRK
1773.919	M	1773.914	0.005	359	374	1	GNEFDELVPDITGLKK
1909.926	M	1909.960	-0.034	214	230	0	MTAADFLVLHFNQDLAK
1920.925	M	1920.978	-0.054	294	311	1	DVDNQTIKETLSAFGGVK
2006.914	M	2006.990	-0.076	388	407	1	AADKAGVAYVEATDIADTR
2210.166	M	2210.164	0.001	59	78	0	VITGGHPLELLDEEFALMVK
2639.367	M	2639.416	-0.048	349	373	1	VVLIAGGLDRGNEFDELVPDITGLK
2860.469	M	2860.474	-0.005	263	291	0	GEVVHAANEIGVPGSHNVENALATIAVAK
2924.471	M	2924.511	-0.040	31	58	0	LGAIVTVNDGKPFEDNPAAQSLLLEEGIK

**FIGURE 109**

SEQ ID NO: 139

ATGGCTAAAGAAACATTTTATATAACAACCCCAATATACTATCCTAGTGGGAATTTACA  
5 TATAGGACATGCATATTCTACAGTGGCTGGAGATGTTATTGCAAGATATAAGAGAATGCAAGGA  
TATGATGTTTCGCTATTTGACTGGAACGGATGAACACGGTCAAAAAATTCAAGAAAAAGCTCAAA  
AAGCTGGTAAGACAGAAATTGAATATTTGGATGAGATGATTGCTGGAATTAAACAATTGTGGGC  
TAAGCTTGAAATTTCAAATGATGATTTTATCAGAACAACCTGAAGAACGTCATAAACATGTCGTTG  
AGCAAGTGTTTGAACGTTTATTAAAGCAAGGTGATATCTATTTAGGTGAATATGAAGGTTGGTAT  
10 TCTGTTCCGGATGAAACATACTATACAGAGTCACAATTAGTAGACCCACAATACGAAAACGGTA  
AAATTATTGGTGGCAAAAGTCCAGATTCTGGACACGAAGTTGAACTAGTTAAAGAAGAAAGTTA  
TTTCTTTAATATTAGTAAATATACAGACCGTTTATTAGAGTTCTATGACCAAAATCCAGATTTTAT  
ACAACCACCATCAAGAAAAAATGAAATGATTAACAACCTTCATTAAACCAGGACTTGCTGATTTA  
GCTGTTTCTCGTACATCATTTAACTGGGGTGTCCATGTTCCGTCTAATCCAAAACATGTTGTTTAT  
15 GTTTGGATTGATGCGTTAGTTAACTATATTTTACGATTAGGCTATTTATCAGATGATGAGTCACT  
ATTTAACAATACTGGCCAGCAGATATTCATTTAATGGCTAAGGAAATTGTGCGATTCCACTCAA  
TTATTTGGCCTATTTTATTGATGGCATTAGACTTACCGTTACCTAAAAAAGTCTTTGCACATGGTT  
GGATTTTGATGAAAGATGGAAAAATGAGTAAATCTAAAGGTAATGTCGTAGACCTAATATTTT  
AATTGATCGCTATGGTTTAGATGCTACACGTTATTATCTAATGCGTGAATTACCATTTGGTTCAG  
20 ATGGCGTATTTACACCTGAAGCATTGTGAGCGTACAAATTTTCGATCTAGCAAATGACTTAGGT  
AACTTAGTAAACCGTACGATTTCTATGGTTAATAAGTACTTTGATGGCGAATTACCAGCGTATCA  
AGGTCCACTTCATGAATTAGATGAAGAAATGGAAGCTATGGCTTTAGAAACAGTGAAAAGCTAC  
ACTGAAAGCATGGAAAGTTTGCAATTTTCTGTGGCATTATCTACGGTATGGAAGTTTATTAGTAG  
AACGAATAAGTATATTGACGAAACAACGCCTTGGGTATTAGCTAAGGACGATAGCCAAAAAGAT  
25 ATGTTAGGCAATGTAATGGCTCACTTAGTTGAAAATATTCGTTATGCAGCTGTATTATTACGTCC  
ATTCTTAACACATGCGCCGAAAGAGATTTTGAACAATTGAACATTAACAATCCTCAATTTATGG  
AATTTAGTAGTTTAGAGCAATATGGTGTGCTTAATGAGTCAATTATGGTTACTGGGCAACCTAAA  
CCTATTTTCCCAAGATTGGATAGCGAAGCGGAAATTGCATATATCAAAGAATCAATGCAACCGC  
CTGCTACTAAAGAGGAAAAAGAAGAGATTCTAGCAAACCTCAAATTGATATTAAAGACTTTGA  
30 TAAAGTTGAAATTAAGGCAGCAACGATTATTGATGCTGAACATGTTAAGAAGTCAGATAAGCTT  
TTAAAAATTCAAGTAGACTTAGATTCTGAACAAAGACAAATTGTATCAGGAATTGCCAAATTCT  
ATACACCAGATGATATTATTGGTAAAAAAGTAGCAGTTGTTACTAACCTGAAACCAGCTAAATT  
AATGGGACAAAAATCTGAAGGTATGATATTATCTGCTGAAAAAGATGGTGTATTAACCTTAGTA  
AGTTTACCAAGTGCAATTCCAAATGGTGCAGTGATTAAATAA

**FIGURE 110**

SEQ ID NO: 140

MAKETFYITTPIIYPSGNLHIGHAYSTVAGDVIARYKRMQGYDVRYLTGTD  
5 EHGQKIQEKAQKAGKTEIEYLDEMIAGIKQLWAKLEISNDDFIRTTEERHKHVVEQ  
VFERLLKQGDIYLGEGWYSVPDETYYESQLVDPQYENGKIIGGKSPDSGHEVE  
LVKEESYFFNISKYTDRLLEFYDQNPDFIQPPSRKNEMINNFIPGLADLAVSRTSFN  
WGVHVPSNPKHVVYVWIDALVNYISALGYLSDDDESLENKYWPADIHLMAKEIVRF  
HSIIWPILLMALDLPLPKKVFAHGWILMKDGKMSKSKGNVVDPNILIDRYGLDATR  
10 YYLMRELPGSDGVFTPEAFVERTNFDLANDLGNLVNRTISMVNKYFDGELPAYQ  
GPLHELDEEMEAMALETVKSYTESMESLQFSVALSTVWKFISRTNKYIDETTPWVL  
AKDDSQKDMLGNVMAHLVENIRYAAVLLRPFLTHAPKEIFEQLNINNPQFMEFSSL  
EQYGVNLNESIMVTGQPKPIFPRLDSEAEIAYIKESMQPPATKEEKEEIPSKPQIDIKDF  
DKVEIKAATIIDAHEHVKKSDKLLKIQVDLDSEQRQIVSGIAKFYTPDDIIGKKVAVVT  
15 NLKPAKLMGQKSEGMILSAEKDGVLTSLPSAIPNGAVIK



**FIGURE 111**

SEQ ID NO: 141

ATGGCTAAAGAAACATTTTATATAACAACCCCAATATACTATCCTAGTGGGAATTTACA  
5 TATAGGACATGCATATTCTACAGTGGCTGGAGATGTTATTGCAAGATATAAGAGAATGCAAGGA  
TATGATGTTTCGCTATTTGACTGGAACGGATGAACACGGTCAAAAAATTCAAGAAAAAGCTCAAA  
AAGCTGGTAAGACAGAAATTGAATATTTGGATGAGATGATTGCTGGAATTAACAATTGTGGGC  
TAAGCTTGAAATTTCAAATGATGATTTTATCAGAACAACCTGAAGAACGTCATAAACATGTCGTTG  
AGCAAGTGTTTGAACGTTTATTAAAGCAAGGTGATATCTATTTAGGTGAATATGAAGGTTGGTAT  
10 TCTGTTCCGGATGAAACATACTATAACAGAGTCACAATTAGTAGACCCACAATACGAAAACGGTA  
AAATTATTGGTGGCAAAAGTCCAGATTCTGGACACGAAGTTGAACTAGTTAAAGAAGAAAGTTA  
TTTCTTTAATATTAGTAAATATACAGACCGTTTATTAGAGTTCTATGACCAAAATCCAGATTTTAT  
ACAACCACCATCAAGAAAAAATGAAATGATTAAACAACCTTCATTAAACCAGGACTTGCTGATTTA  
GCTGTTTCTCGTACATCATTTAACTGGGGTGTCCCTGTTCCGTCTAATCCAAAACATGTTGTTTAT  
15 GTTTGGATTGATGCGTTAGTTAACTATATTTGAGCATTAGGCTATTTATCAGATGATGAGTCACT  
ATTTAACAAATACTGGCCAGCAGATATTCATTTAATGGCTAAGGAAATTGTGCGATTCCACTCAA  
TTATTTGGCCTATTTTATTGATGGCATTAGACTTACCGTTACCTAAAAAGTCTTTGCACATGGTT  
GGATTTTGATGAAAGATGGAAAAATGAGTAAATCTAAAGGTAATGTCGTAGACCCTAATATTTT  
AATTGATCGCTATGGTTTAGATGCTACACGTTATTATCTAATGCGTGAATTACCATTTGGTTCAG  
20 ATGGCGTATTTACACCTGAAGCATTTGTTGAGCGTACAAATTTTCGATCTAGCAAATGACTTAGGT  
AACTTAGTAAACCGTACGATTTCTATGGTTAATAAGTACTTTGATGGCGAATTACCAGCGTATCA  
AGGTCCACTTCATGAATTAGATGAAGAAATGGAAGCTATGGCTTTAGAAACAGTGAAAAGCTAC  
ACTGAAAGCATGGAAAGTTTGCAATTTTCTGTGGCATTATCTACGGTATGGAAGTTTATTAGTAG  
AACGAATAAGTATATTGACGAAACAACCCCTTGGGTATTAGCTAAGGACGATAGCCAAAAAGAT  
25 ATGTTAGGCAATGTAATGGCTCACTTAGTTGAAAATATTCGTTATGCAGCTGTATTATTACGTCC  
ATTCTTAACACATGCGCCGAAAGAGATTTTTGAACAATTGAACATTAACAATCCTCAATTTATGG  
AATTTAGTAGTTTAGAGCAATATGGTGTGCTTAATGAGTCAATTATGGTTACTGGGCAACCTAAA  
CCTATTTTCCCAAGATTGGATAGCGAAGCGGAAATTGCATATATCAAAGAATCAATGCAACCGC  
CTGCTACTAAAGAGGAAAAAGAAGAGATTCTAGCAAACCTCAAATTGATATTAAGACTTTGA  
30 TAAAGTTGAAATTAAGGCAGCAACGATTATTGATGCTGAACATGTTAAGAAGTCAGATAAGCTT  
TTAAAAATTCAAGTAGACTTAGATTCTGAACAAAGACAAATTGTATCAGGAAATGCCAAATTCT  
ATACACCAGATGATATTATTGGTAAAAAAGTAGCAGTTGTTACTAACCTGAAACCGGCTAAATT  
AATGGGACAAAAATCTGAAGGTATGATATTATCTGCTGAAAAAGATGGTGTATTAACCTTAGTA  
AGTTTACCAAGTGCAATTCCAAATGGTGCAGTGATTAAATAA

**FIGURE 112**

SEQ ID NO: 142

MAKETFYITTPIIYYPSGNLHIGHAYSTVAGDVIARYKRMQGYDVRYLTGTD  
5 EHGQKIQEKAQKAGKTEIEYLDEMIAGIKQLWAKLEISNDDFIRTTEERHKHVVEQ  
VFERLLKQGDIYLG EYEGWYSVPDETYYESQLVDPQYENGKIIGGKSPDSGHEVE  
LVKEESYFFNISKYTDRLLEFYDQNPDFIQPPSRKNEMINNFIKPGLADLAVSRTSFN  
WGVPVPSNPKHVYVWIDALVNYISALGYLSDDDESLENKYWPADIHLMAKEIVRF  
HSIIWPILLMALDLPLPKKVFAHGWILMKDGKMSKSKGNVVDPNILIDRYGLDATR  
10 YYLMREL PFGSDGVFTPEAFVERTNFDLANDLG NLVNRTISMVNKYFDGELPAYQ  
GPLHELDEEMEAMALET VKSYTESMESLQFSVALSTVWK FISRTN KYIDETTPWVL  
AKDDSQKDMLGNVMAHLVENIRYAAVLLRPFLTHAPKEIFEQLNINNPQFMEFSSL  
EQYGVLNESIMVTGQPKPIFPRLDSEAEIAYIKESMQPPATKEEKEEIPSKPQIDIKDF  
DKVEIKAATIIDA EHVKKSDKLLKIQVDLDSEQRQIVSGIAKFYTPDDIIGKKVAVVT  
15 NLKPAKLMGQKSEGMILSAEKDGVLT LVSLPSAIPNGAVIK

**FIGURE 113**

SEQ ID NO: 143

Forward PCR Primer

5 GCGGCGGCCCATATGAGTACATTAGAACAACAATAG

SEQ ID NO: 144

10

Reverse PCR Primer

GCGCGGATCCTTAATAGCCTTTCAGCGCGGC

**FIGURE 114****TABLE 21 Properties of methionyl-tRNA synthetase from *S. aureus***

TABLE 21 -- methionyl-tRNA synthetase from <i>S. aureus</i> -- SEQ ID NO: 139-SEQ ID NO: 142	
Melting temperature (°C) of SEQ ID NO: 143 (forward PCR primer)	64
Restriction enzyme for SEQ ID NO: 143 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 144 (reverse PCR primer)	58
Restriction enzyme for SEQ ID NO: 144 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 139	1974
Number of amino acid residues in SEQ ID NO: 140	657
Number of different nucleic acid residues between SEQ ID NO: 139 and SEQ ID NO: 141	3
Number of different amino acid residues between SEQ ID NO: 140 and SEQ ID NO: 142	1
Calculated molecular weight of SEQ ID NO: 140 polypeptide (kDa)	73.1
Calculated pI of SEQ ID NO: 140 polypeptide	4.8
Solubility of SEQ ID NO: 142 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching 100%
Amount of purified polypeptide having SEQ ID NO: 142, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	4.9
Amount of purified polypeptide having SEQ ID NO: 142 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	10.9
Z-score for the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 142, determined as described in EXAMPLE 9	1.7E-10
Number of matched peptides in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 142, determined as described in EXAMPLE 9	28
Minimum sequence coverage in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 142, determined as described in EXAMPLE 9	46
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. No interacting proteins were observed by using at least one of the methods described in those examples.	

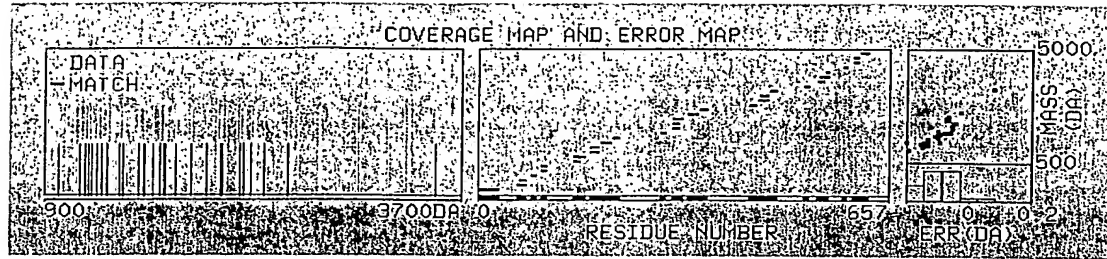
FIGURE 115

TABLE 22 Bioinformatic Analyses of methionyl-tRNA synthetase from *S. aureus*

TABLE 22 -- methionyl-tRNA synthetase from <i>S. aureus</i> -- SEQ ID NO: 139-SEQ ID NO: 142	
COG Category	Translation, ribosomal structure, and biogenesis
COG ID Number	COG0143
Is SEQ ID NO: 140 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 140	Methionyl-tRNA Synthetase, (1a8h)
Source organism for closest PDB protein to SEQ ID NO: 140	<i>Thermus aquaticus</i>
e-value for closest PDB Protein to SEQ ID NO: 140	1E-102
% Identity between SEQ ID NO: 140 and the closest protein from PDB	38
% Positives between SEQ ID NO: 140 and the closest protein from PDB	57
Number of Protein Hits in the VGDB to SEQ ID NO: 140	14
Number of Microorganisms having VGDB Hits to SEQ ID NO: 140	11
Microorganisms having VGDB Hits to SEQ ID NO: 140 <sup>1</sup>	[saur][bsub][efae][spne][ecoli][hpyl][rpxx][mgen][bbur][paer][nmen]
First predicted epitopic region of SEQ ID NO: 140: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 145 :WGVHVPSNPKHVVYVW-IDALVNYISALGYL, 1.209,222->251
Second predicted epitopic region of SEQ ID NO: 140: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 146 :DGVLTSLVSLPSAIPNGA, 1.205,638->654
Third predicted epitopic region of SEQ ID NO: 140: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 147 :HKHVVEQVFERLLKQG-DIYLGE, 1.173,101->122

- <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Eschericia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpxx* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

FIGURE 116



Note: click on the ☐ symbol to change column format.

Measured	Avg/	Computed	Error	<input type="checkbox"/> Residues	Missed		
Mass (M)	Mono	Mass	(Da)	Start	To	Cut	Peptide sequence
992.327	M	992.517	-0.190	557	564	1	DFDKVEIK
1141.443	M	1141.588	-0.145	103	111	0	HVVEQVFER
1167.437	M	1167.581	-0.144	601	610	0	FYTPDDIIGK
1201.463	M	1201.593	-0.131	583	592	0	IQVDLDSEQR
1220.475	M	1220.603	-0.129	86	95	0	LEISNDDFIR
1250.487	M	1250.639	-0.152	522	532	0	LDSEAEIAYIK
1295.544	M	1295.635	-0.091	155	166	0	SPDSGHEVELVK
1295.544	M	1295.675	-0.131	601	611	1	FYTPDDIIGKK
1323.571	M	1323.714	-0.143	314	325	0	GNVVDPNILIDR
1328.543	M	1328.742	-0.199	295	305	1	KVFAHGWLIMK
1406.612	M	1406.741	-0.129	101	111	1	HKHVVEQVFER
1434.608	M	1434.739	-0.131	434	445	0	YIDETTPWVLAK
1538.746	M	1538.841	-0.095	312	325	1	SKGNVVDPNILIDR
1568.667	M	1568.773	-0.106	218	231	0	TSPNWGVHVPSPK
1623.713	M	1623.806	-0.093	67	80	0	TEIEYLDEMIAGIK
1674.777	M	1674.832	-0.055	356	370	0	TNFDLANDLGNLVNR
1695.909	M	1696.006	-0.097	612	627	1	VAVVTNLKPAKLMGQK
1695.909	M	1695.982	-0.073	466	480	0	YAAVLLRPFLTHAPK
1710.778	M	1710.854	-0.076	451	465	0	DMLGNVMAHLVENIR
1781.844	M	1781.940	-0.096	542	556	1	EEKKEEIPSKPQIDIK
1879.906	M	1879.959	-0.053	64	80	1	AGKTEIEYLDEMIAGIK
1995.904	M	1995.957	-0.053	338	355	0	ELPFGSDGVFTPEAFVER
2077.965	M	2078.010	-0.045	181	197	0	LLEFYDQNPDIQPPSR
2100.053	M	2100.095	-0.042	314	332	1	GNVVDPNILIDRYGLDTR
2206.039	M	2206.105	-0.066	181	198	1	LLEFYDQNPDIQPPSRK
2229.123	M	2229.193	-0.070	198	217	1	KNEMINNFIFKPLADLAVSR
2284.021	M	2284.093	-0.072	446	465	1	DDSQKMDMLGNVMAHLVENIR
2370.206	M	2370.267	-0.061	545	564	2	EEIPSKPQIDIKDFDKVEIK
2540.182	M	2540.206	-0.024	155	176	1	SPDSGHEVELVKESYFFNISK
3525.835	M	3525.755	0.080	4	35	0	ETFYITPTPIYYPSGNLHIGHAYSTVAGDVIAR

**FIGURE 117**

SEQ ID NO: 148

ATGACGAATGTATTAATTGAAGATTTAAAATGGAGAGGTCTTATTTATC  
5 AACAAACTGATGAACAAGGTATTGAAGATTTATTAAATAAAGAACAAGTGACG  
TTATACTGCGGTGCCGATCCAACGGCAGATAGTTTACATATTGGTCACTTACTA  
CCATTCTTAACATTAAGACGTTTTCAAGAACATGGACATCGTCCTATCGTTTTA  
ATTGGCGGTGGTACAGGTATGATTGGTGATCCATCAGGTAAATCAGAAGAACG  
TGTGCTACAAACAGAAGAACAAGTAGATAAAAAATATCGAAGGTATTAGTAAGC  
10 AAATGCACAATATTTTTGAATTTGGAACAGACCATGGTGCAGTGCTTGTTAATA  
ATAGAGACTGGTTAGGACAAATCTCATTAAATTAGTTTTTTACGTGACTATGGTA  
AACACGTCGGCGTTAATTACATGTTAGGTAAAGATTCAATCCAAAGTCGTTTAG  
AACATGGTATTTTCATATACAGAATTCACATACACGATTTTACAAGCTATTGATT  
TCGGTCATTTGAATAGAGAATTGAATTGTAAGATTCAAGTAGGTGGATCAGATC  
15 AATGGGGTAATATCACAGTGGTATTGAATTAATGCGTCGTATGTATGGTCAAA  
CAGACGCATACGGTTTAACTATTCCGCTTGTAATAATCAGATGGTAAGAAAT  
TTGGTAAGTCTGAGTCAGGTGCTGTTTGGTTAGATGCTGAAAAACAAGTCCTT  
ATGAATTTTATCAATTCTGGATTAATCAATCAGACGAAGATGTAATTAATTCT  
TAAAATACTTTACTTTCTTAGGAAAAGAAGAAATTGATCGCTTAGAACAATCTA  
20 AAAATGAAGCACCGCATTTACGTGAAGCTCAAAAAACATTAGCTGAAGAAGTA  
ACTAAATTTATTCATGGTGAAGATGCATTAAATGATGCAATCCGTATTTACAA  
GCATTATTTAGTGGTGATTTAAAATCATTATCAGCGAAAGAATTAAGATGGA  
TTTAAAGATGTGCCTCAAGTGACATTATCAAATGACACAACAAATATCGTTGAA  
GTCCTTATTGAAACAGGCATTTCTCCTTCTAAACGACAAGCACGTGAAGATGTT  
25 AACAATGGTGCGATTTATATTAATGGTGAGAGACAACAAGATGTTAATTATGCT  
TTAGCACCAGAAGATAAAATTGATGGCGAATTTACGATTATTCGTCGCGGTAAG  
AAAAAATACTTCATGGTTAACTATCAATAA

**FIGURE 118**

SEQ ID NO: 149

MTNVLIEDLKWRGLIYQQTDEQGIEDLLNKEQVTLYCGADPTADSLHIGHL  
5 LPFLTLLRRFQEHGHRPIVLIGGGTGMIGDPSGKSEERVVLQTEEQVDKNIEGISKQMH  
NIFEFGTDHGAVLVNNRDWLQISLISFLRDYGKHVGVNYMLGKDSIQSRLEHGIS  
YTEFTYITILQAIDFGHLNRELNCKIQVGGSDQWGNITSGIELMRRMYGQTDAYGLT  
IPLVTKSDGKKFGKSESGAVWLDAEKTSPYEFYQFWINQSDDEVKFLKYFTFLGK  
EEDRLEQSKNEAPHLREAQKTLAEVTKFIHGEDALNDAIRISQALFSGDLKSLSA  
10 KELKDGFKDVPQVTLSTNDTTNIVEVLIETGISPSKRQAREDVNNGAIYINGERQQDV  
NYALAPEDKIDGFTIIRRGKKKYFMVNYQ



**FIGURE 119**

SEQ ID NO: 150

ATGACGAATGTATTAATTGAAGATTTAAAATGGAGAGGTCTTATTTATC  
5 AACAAACTGATGAACAAGGTATTGAAGATTTATTAAATAAAGAACAAGTGACG  
TTATACTGCGGTGCCGATCCAACGGCAGATAGTTTACATATTGGTCACTTACTA  
CCTTTCTTAACATTAAGACGTTTTTCAAGAACATGGACATCGTCCTATCGTTTTAA  
TTGGCGGTGGTACTGGTATGATTGGTGATCCATCAGGTAAATCAGAAGAACGT  
GTGCTACAAACAGAAGAACAAGTAGATAAAAAATATCGAAGGTATTAGTAAGCA  
10 AATGCACAATATTTTTGAATTTGGAACAGACCATGGTGCAGTGCTTGTTAATAA  
TAGAGACTGGTTAGGACAAATCTCATTAAATTAGTTTTTTACGTGACTATGGTAA  
ACACGTCGGCGTTAATTACATGTTAGGTAAAGATTCAATCCAAAGTCGTTTAGA  
ACATGGTATTTTCATATACAGAATTCACATACACGATTTTACAAGCTATTGATTT  
CGGTCATTTGAATAGAGAATTGAATTGTGAGATTCAAGTAGGTGGATCAGATC  
15 AATGGGGTAATATCACAAGTGGTATTGAATTAATGCGTCGTATGTATGGTCAAA  
CAGACGCATACGGTTTAACTATTCCGCTTGTAATAATCAGATGGTAAGAAAT  
TTGGTAAGTCTGAGTCAGGTGCTGTTTGGTTAGATGCTGAAAAACAAGTCCTT  
ATGAATTTTATCAATTCTGGATTAATCAATCAGACGAAGATGTAATTAAATTCT  
TAAAATACTTTACTTTCTTAGGAAAAGAAGAAATTGATCGCTTAGAACAATCTA  
20 AAAATGAAGCACCGCATTACGTGAAGCTCAAAAAACATTAGCTGAAGAAGTA  
ACTAAATTTATTCATGGTGAAGATGCATTAAATGATGCAATCCGTATTTCAAA  
GCATTATTTAGTGGTGATTTAAAATCATTATCAGCGAAAGAATTAAGATGGG  
TTTAAAGATGTGCCTCAAGTGACATTATCAAATGACACAACAAATATCGTTGAA  
GTCCTTATTGAAACAGGCATTTCTCCTTCTAAACGACAAGCACGTGAAGATGTT  
25 AACAATGGTGCGATTTATATTAATGGTGAGAGACAACAAGATGTTAATTATGCT  
TTAGCACCAGAAGATAAAATTGATGGCGAATTTACGATTATTCGTCGCGGTAAG  
AAAAAATACTTCATGGTTAACTATCAATAA

**FIGURE 120**

SEQ ID NO: 151

MTNVLIEDLKWRGLIYQQTDEQGIEDLLNKEQVTLYCGADPTADSLHIGHL  
5 LPFLTLLRRFQEHGHRPIVLIGGGTGMIGDPSGKSEERVVLQTEEQVDKNIEGISKQMH  
NIFEFGTDHGAVLVNNRDWLGGQISLISFLRDYGKHVGVNYMLGKDSIQRLEHGIS  
YTEFTYITILQAIDFGHLNRELNCEIQVGGSDQWGNITSGIELMRRMYGQTDAYGLTI  
PLVTKSDGKKFGKSESGAVWLDAEKTSPYEFYQFWINQSDDEVKFLKYFTFLGKE  
EIDRLEQSKNEAPHLREAQKTLAEVTKFIHGEDALNDAIRISQALFSGDLKSLSAK  
10 ELKDGFKDVPQVTLSNDTTNIVEVLIETGISPSKRQAREDVNNGAIYINGERQQDVN  
YALAPEDKIDGEFTIIRRGKKKYFMVNYQ

**FIGURE 121**

SEQ ID NO: 152

Forward PCR Primer

5 GCGGCGGCCCATATGGGCACGACCAAACACAG

SEQ ID NO: 153

10

Reverse PCR Primer

GCGCGGATCCTTAGATATGATCAAAAATGATCTCAG

**FIGURE 122****TABLE 23 Properties of tyrosyl-tRNA synthetase from *S. aureus***

TABLE 23 -- tyrosyl-tRNA synthetase from <i>S. aureus</i> -- SEQ ID NO: 148-SEQ ID NO: 151	
Melting temperature (°C) of SEQ ID NO: 152 (forward PCR primer)	62
Restriction enzyme for SEQ ID NO: 152 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 153 (reverse PCR primer)	60
Restriction enzyme for SEQ ID NO: 153 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 148	4
Number of amino acid residues in SEQ ID NO: 149	1
Number of different nucleic acid residues between SEQ ID NO: 148 and SEQ ID NO: 150	1263
Number of different amino acid residues between SEQ ID NO: 149 and SEQ ID NO: 151	420
Calculated molecular weight of SEQ ID NO: 149 polypeptide (kDa)	46.8
Calculated pI of SEQ ID NO: 149 polypeptide	4.9
Solubility of SEQ ID NO: 151 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching 100%
Amount of purified polypeptide having SEQ ID NO: 151, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	11.8
Amount of purified polypeptide having SEQ ID NO: 151 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	24.9
Z-score for the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 151, determined as described in EXAMPLE 9	6.8E-06
Number of matched peptides in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 151, determined as described in EXAMPLE 9	22
Minimum sequence coverage in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 151, determined as described in EXAMPLE 9	66
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. The identity of interacting proteins identified by using at least one of the methods described in those examples are: 45 and 66 kDa unidentified proteins.	

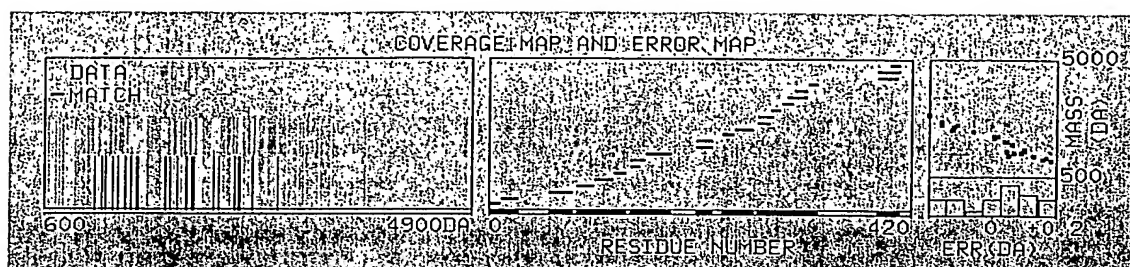
FIGURE 123

TABLE 24 Bioinformatic Analyses of tyrosyl-tRNA synthetase from *S. aureus*

TABLE 24 -- tyrosyl-tRNA synthetase from <i>S. aureus</i> -- SEQ ID NO: 148-SEQ ID NO: 151	
COG Category	Translation, ribosomal structure, and biogenesis
COG ID Number	COG0162
Is SEQ ID NO: 149 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 149	Tyrosyl-tRNA Synthetase (1jil)
Source organism for closest PDB protein to SEQ ID NO: 149	<i>Staphylococcus aureus</i>
e-value for closest PDB Protein to SEQ ID NO: 149	0
% Identity between SEQ ID NO: 149 and the closest protein from PDB	100
% Positives between SEQ ID NO: 149 and the closest protein from PDB	100
Number of Protein Hits in the VGDB to SEQ ID NO: 149	16
Number of Microorganisms having VGDB Hits to SEQ ID NO: 149	13
Microorganisms having VGDB Hits to SEQ ID NO: 149 <sup>1</sup>	[saur][bsub][efae][spne][ecoli][nmen][bbur][rpxx][ctra][paer][mgen][hinf][hpyl]
First predicted epitopic region of SEQ ID NO: 149: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 154 :ADSLHIGHLLPFLTLR, 1.152,43->58
Second predicted epitopic region of SEQ ID NO: 149: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 155 :KEQVTLYCGAD, 1.15,30->40
Third predicted epitopic region of SEQ ID NO: 149: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 156 :IVEVLIETG, 1.146,355->363

- <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Eschericia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpxx* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

FIGURE 124



Note: click on the ☐ symbol to change column format.

Measured	Avg/	Computed	Error	<input type="checkbox"/> Residues	Missed	
Mass (M)	Mono	Mass	(Da)	Start	To	Peptide sequence
1116.739	M	1116.574	0.165	143	152	0 HVGVNMYLKG
1177.772	M	1177.634	0.139	319	329	0 ISQALFSGDLK
1218.822	M	1218.672	0.151	400	409	1 IDGEFTIIRR
1290.722	M	1290.609	0.114	235	246	0 SESGAVWLDAEK
1345.756	M	1345.708	0.048	294	305	1 EAQKTLAEVTK
1420.802	M	1420.742	0.060	282	293	1 LEQSKNEAPHLR
1469.807	M	1469.726	0.081	306	318	0 FIHGEDALNDAIR
1516.847	M	1516.755	0.092	270	281	1 YFTFLGKEEIDR
1516.847	M	1516.807	0.041	1	12	1 MTNVLIEDLKWR
1546.940	M	1546.850	0.090	126	138	0 DWLGQISLISFLR
1802.967	M	1802.909	0.058	143	158	1 HVGVNMYLKGKDSIQSR
1869.996	M	1869.954	0.042	210	226	0 MYGQTDAYGLTIPLVTK
1929.043	M	1929.004	0.038	89	105	1 VLQTEEQVDKNIEGISK
2026.061	M	2026.055	0.007	209	226	1 RMYGQTDAYGLTIPLVTK
2076.044	M	2076.036	0.008	13	30	0 GLIYQQTDEQGIEDLLNK
2102.083	M	2102.067	0.016	270	286	2 YFTFLGKEEIDRLEQSK
2298.045	M	2298.095	-0.050	106	125	0 QMHNIFEFGTDHGAVLVNRR
2341.080	M	2341.190	-0.110	298	318	1 TLAEVTKFIHGEDALNDAIR
2508.041	M	2508.147	-0.107	247	266	0 TSPYEFYQFWINQSDDEVK
2534.124	M	2534.264	-0.140	387	408	1 QQDVNYALAPEDKIDGEFTIIR
2559.207	M	2559.300	-0.094	60	84	0 FQEHGHRPIVLIGGGTGMIGDPSGK
2690.226	M	2690.365	-0.139	387	409	2 QQDVNYALAPEDKIDGEFTIIRR
2937.288	M	2937.465	-0.177	159	183	0 LEHGISYTEFTYTILQAIDFGHLNR

**FIGURE 125**

SEQ ID NO: 157

ATGATTAAAATACCTAGAGGGACGCAGGATATTTTACCTGAAGATTCAA  
5 AGAAATGGCGTTACATTGAAAATCAATTAGATGAATTAATGACATTTTATAATT  
ATAAAGAAATAAGAACACCAATTTTTGAAAGTACAGATCTTTTTGCAAGAGGT  
GTTGGTGATTCAACCGATGTCGTACAAAAAGAAATGTATACATTTAAAGATAA  
AGGCGATAGAAGTATTACATTAAGACCTGAGGGAACAGCTGCAGTTGTGCGTT  
CATATATTGAACATAAAATGCAAGGTAATCCAAACCAACCAATTAAACTTTATT  
10 ACAATGGACCGATGTTTAGATATGAACGTAAGCAAAAAGGACGCTATCGTCAA  
TTAATCAATTTGGTGTAGAAGCTATTGGTGCTGAAAATCCTAGCGTAGATGCA  
GAAGTATTAGCTATGGTTATGCATATTTATCAATCATTTGGATTAAAACATTTA  
AAGCTTGTTATTAATAGTGTAGGGGATATGGCGTCTCGAAAAGAATATAACGA  
AGCGTTAGTGAAACACTTTGAACCAGTAATTCATGAATTTTGTTTCAGATTGTCA  
15 ATCACGTTTGCATACAAATCCGATGCGAATTTTGATTGTAAAGTAGACCGTGA  
TAAAGAAGCGATTAAGACTGCACCTAGAATCACTGATTTCTTAAATGAGGAAT  
CTAAGGCATATTATGAACAAGTAAAAGCTTATTTAGATGATTTAGGTATTCCAT  
ATATTGAAGATCCTAACTTAGTTCGTGGATTGGATTATTATACACATACAGCAT  
TTGAATTAATGATGGATAACCCTAACTATGATGGTGCCATTACAACGCTTTGTG  
20 GTGGTGGCCGTTATAATGGTTTATTAGAATTGCTAGATGGTCCAAGTGAAACAG  
GTATTGGTTTTGCGCTAAGTATAGAACGATTATTGCTTGCACTTGAAGAAGAAG  
GTATCGAATTAGATATTGAAGAAAACCTTAGATTTATTCATTGTTACAATGGGTG  
ATCAAGCAGATCGATATGCTGTGAAGCTATTAAATCATTTGAGACATAATGGTA  
TTAAAGCAGATAAAGACTATTTACAGCGTAAAATTAAAGGACAAATGAAACAA  
25 GCAGACCGTTTAGGTGCCAAGTTTACAATCGTTATTGGTGATCAAGAATTAGAA  
AATAATAAAATCGATGTTAAAAATATGACAACTGGTGAATCTGAAACAATTGA  
ATTAGACGCATTAGTCGAATATTTTAAGAAGTAG

**FIGURE 126**

SEQ ID NO: 158

MIKIPRGTQDILPEDSKKWRYIENQLDELMTFYNYKEIRTPIFESTDLFARGV  
5 GDSTDVVQKEMYTFKDKGDRSITLRPEGTAAVVRSYIEHKMQGNPNQPIKLYYNG  
PMFRYERKQKGRYRQFNQFGVEAIGAENPSVDAEVLAMVMHIYQSFGCLKHLKLV  
NSVGDMASRKEYNEALVKHFEPVIHEFCSDCQSRHTNPMRILDCKVDRDKEAIKT  
APRITDFLNEESKAYYEQVKAYLDDLGPYIEDPNLVRGLDYTHYTHAFELMMDNPN  
YDGAITTLGGRYNGLELLDGPSETGIGFALSIERLLALEEEGIELDIEENLDLFI  
10 VTMGDQADRYAVKLLNHLRHNGIKADKDYLRKIKGQMKQADRLGAKFTIVIGD  
QELNNKIDVKNMTTGESETIELDALVEYFKK



**FIGURE 127**

SEQ ID NO: 159

ATGATTAAAATACCTAGAGGGACGCAGGATATTTTACCTGAAGATTCAA  
5 AGAAATGGCGTTACATTGAAAATCAATTAGATGAATTAATGACATTTTATAATT  
ATAAAGAAATAAGAACACCAATTTTTGAAAGTACAGATCTTTTTGCAAGAGGT  
GTTGGTGATTCAACCGATGTCGTACAAAAAGAAATGTATACATTTAAAGATAA  
AGGCGATAGAAGTATTACATTAAGATCTGAAGGAACAGCTGCAGTTGTGCGTT  
CATATATTGAACATAAAATGCAAGGTAATCCAAACCAACCAATTAAACTTTATT  
10 ACAATGGACCGATGTTTAGATATGAACGTAAGCAAAAAGGACGCTATCGTCAA  
TTAATCAATTTGGTGTAGAAGCTATTGGTGCTGAAAATCCTAGCGTAGATGCA  
GAAGTATTAGCTATGGTTATGCATATTTATCAATCATTGATTAAACATTTA  
AAGATTGTTATTAATAGTGTAGGGGATATGGCGTCTCGAAAAGAATATAACGA  
AGCGTTAGTGAAACACTTTGAACCAGTAATTCATGAATTTTGTTTCAGATTGTCA  
15 ATCACGTTTGCATACAAATCCGATGCGAATTTTGATTGTAAAGTAGACCGTGA  
TAAAGAAGCGATTAAGACTGCACCTAGAATCACTGATTTCTTAAATGAGGAAT  
CTAAGGCATATTATGAACAAGTAAAAGCTTATTTAGATGATTTAGGTATTCCAT  
ATATTGAAGATCCTAACTTAGTTCGTGGATTGGATTATTATACACATACAGCAT  
TTGAATTAATGATGGATAACCCTAACTATGATGGTGCCATTACAACGCTTTGTG  
20 GTGGTGGCCGTTATAATGGTTTATTAGAATTGCTAGATGGTCCAAGTGAAACAG  
GTATTGGTTTTTGCCTAAGTATAGAACGATTATTGCTTGCACTTGAAGAAGAAG  
GTATCGAATTAGATATTGAAGAAAACTTGGATTTATTCATTGTTACAATGGGTG  
ATCAAGCAGATCGATATGCTGTGAAGCTATTAAATCATTTGAGACATAATGGTA  
TTAAAGCAGATAAAGACTATTTACAGCGTAAAATTAAAGGACAAATGAAACAA  
25 GCAGACCGTTTAGGTGCCAAGTTTACAATCGTTATTGGTGATCAAGAATTAGAA  
AATAATAAAATCGATGTTAAAAATATTACAACCTGGTGAATCTGAAACAATTGA  
ATTAGACGCATTAGTCGAATATTTTAAGAAGTAG

**FIGURE 128**

SEQ ID NO: 160

MIKIPRGTQDILPEDSKKWRYIENQLDELMTFYNYKEIRTPIFESTDLFARGV  
5 GDSTDVVQKEMYTFKDKGDRSITLRSEGTAAVVRSYIEHKMQGNPNQPIKLYYNG  
PMFRYERKQKGRYRQFNQFGVEAIGAENPSVDAEVLAMVMHIYQSFGCLKHLKIVI  
NSVGDMASRKEYNEALVKHFEPVIHEFCSDCQSRLHTNPMRILDCKVDRDKEAIKT  
APRITDFLNEESKAYYEQVKAYLDDLGPYIEDPNLVRGLDYYTHTAFELMMDNPN  
YDGAITTLCGGGRYNGLLELLDGPSETGIGFALSIERLLLALEEEGIELDIEENLDLFI  
10 VTMGDQADRYAVKLLNHLRHNGIKADKDYLRKIKGQMKQADRLGAKFTIVIGD  
QELNNKIDVKNITTGESETIELDALVEYFKK

**FIGURE 129**

SEQ ID NO: 161

Forward PCR Primer

5 GCGGCGGCCCATATGGCTCGTACAACACCCATC

SEQ ID NO: 162

10

Reverse PCR Primer

GCGCGGATCCTTATTATTACCACGGGCTTCAATTAC

**FIGURE 130****TABLE 25 Properties of histidyl-tRNA synthetase from *S. aureus***

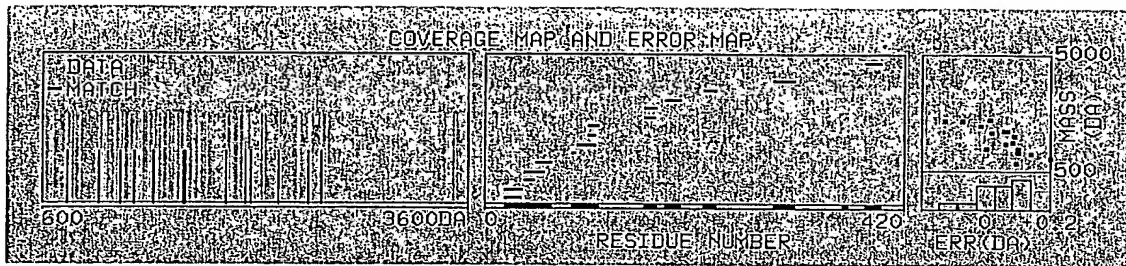
TABLE 25 -- histidyl-tRNA synthetase from <i>S. aureus</i> -- SEQ ID NO: 157-SEQ ID NO: 160	
Melting temperature (°C) of SEQ ID NO: 161 (forward PCR primer)	64
Restriction enzyme for SEQ ID NO: 161 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 162 (reverse PCR primer)	66
Restriction enzyme for SEQ ID NO: 162 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 157	1263
Number of amino acid residues in SEQ ID NO: 158	420
Number of different nucleic acid residues between SEQ ID NO: 157 and SEQ ID NO: 159	5
Number of different amino acid residues between SEQ ID NO: 158 and SEQ ID NO: 160	3
Calculated molecular weight of SEQ ID NO: 158 polypeptide (kDa)	46.8
Calculated pI of SEQ ID NO: 158 polypeptide	4.9
Solubility of SEQ ID NO: 160 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching 100%
Amount of purified polypeptide having SEQ ID NO: 160, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	18.7
Amount of purified polypeptide having SEQ ID NO: 160 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	35.6
Z-score for the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 160, determined as described in EXAMPLE 9	1.1E-05
Number of matched peptides in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 160, determined as described in EXAMPLE 9	15
Minimum sequence coverage in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 160, determined as described in EXAMPLE 9	37
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. No interacting proteins were observed by using at least one of the methods described in those examples.	

**FIGURE 131****TABLE 26 Bioinformatic Analyses of histidyl-tRNA synthetase from *S. aureus***

TABLE 26 -- histidyl-tRNA synthetase from <i>S. aureus</i> -- SEQ ID NO: 157-SEQ ID NO: 160	
COG Category	Translation, ribosomal structure, and biogenesis
COG ID Number	COG0124
Is SEQ ID NO: 158 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 158	Histidyl-tRNA Synthetase (1qe0)
Source organism for closest PDB protein to SEQ ID NO: 158	<i>Staphylococcus aureus</i>
e-value for closest PDB Protein to SEQ ID NO: 158	0
% Identity between SEQ ID NO: 158 and the closest protein from PDB	99
% Positives between SEQ ID NO: 158 and the closest protein from PDB	99
Number of Protein Hits in the VGDB to SEQ ID NO: 158	11
Number of Microorganisms having VGDB Hits to SEQ ID NO: 158	11
Microorganisms having VGDB Hits to SEQ ID NO: 158 <sup>1</sup>	[spne][bsub][hinf][ecoli][efae][paer][saur][nmen][ctra][rpxx][mgen]
First predicted epitopic region of SEQ ID NO: 158: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 163 :NPSVDAEVLAMVMH-IYQSFGLKHLKLVINS, 1.157,136->165
Second predicted epitopic region of SEQ ID NO: 158: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 164 :EALVKHFEFVIHEFCS-DCQSR, 1.151,177->197
Third predicted epitopic region of SEQ ID NO: 158: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 165 :TAAVVRSYIEH, 1.147,82->92

- <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Eschericia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpxx* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

FIGURE 132



Note: click on the ☐ symbol to change column format.

Measured Mass (M)	Avg/ Mono	Computed Mass	Error (Da)	<div><div></div>Residues</div>	Missed	Peptide sequence	
				Start	To	Cut	
775.471	M	775.386	0.085	88	93	0	SYIEHK
1007.677	M	1007.503	0.174	359	366	1	ADKDYLR
1159.672	M	1159.548	0.124	104	112	0	LYYNGPMFR
1260.741	M	1260.649	0.092	161	172	0	LVINSVGDMASR
1388.746	M	1388.744	0.002	161	173	1	LVINSVGDMASRK
1395.792	M	1395.703	0.089	40	51	0	TPIFESTDLFAR
1607.809	M	1607.755	0.054	104	115	1	LYYNGPMFRYER
1619.889	M	1619.815	0.074	219	232	1	TAPRITDFLNEESK
1902.983	M	1902.902	0.081	52	68	1	GVGDSTDVVQKEMYTFK
2046.884	M	2046.867	0.018	182	197	0	HFEPVTHEFCSDCQSR
2074.142	M	2074.094	0.048	382	399	1	FTIVIGDQLENNKIDVK
2083.020	M	2082.960	0.060	21	36	0	YIENQLDELMTFYNYK
2267.085	M	2267.097	-0.012	94	112	1	MQGNENQPIKLYYNGPMFR
2481.129	M	2481.188	-0.059	21	39	1	YIENQLDELMTFYNYKEIR
2481.129	M	2481.238	-0.109	40	62	1	TPIFESTDLFARGVGSTDVVQK
2563.330	M	2563.315	0.014	289	312	0	YNGLELLDGPSETGIGFALSIER

**FIGURE 133**

SEQ ID NO: 166

TTGAGGATGAATAAAAATGTCAGCTTTTATAACTTTTGAGGGCCCAGAAG  
5 GCTCTGGAAAAACAACCTGTAATTAATGAAGTTTACCATAGATTAGTAAAAGATT  
ATGATGTCATTATGACTAGAGAACCAGGTGGTGTTCCTACTGGTGAAGAAATAC  
GTAAAATTGTATTAGAAGGCAATGATATGGACATTAGAACTGAAGCAATGTTA  
TTTGCTGCATCTAGAAGAGAACATCTTGTATTAAAGGTCATACCAGCTTTAAAA  
GAAGGTAAGGTTGTGTTGTGTGATCGCTATATCGATAGTTCATTAGCTTATCAA  
10 GGTTATGCTAGAGGGATTGGCGTTGAAGAAGTAAGAGCATTAAACGAATTTGC  
AATAAATGGATTATATCCAGACTTGACGATTTATTTAAATGTTAGTGCTGAAGT  
AGGTCGCGAACGTATTATTAAAAATTCAAGAGATCAAAATAGATTAGATCAAG  
AAGATTTAAAGTTTCACGAAAAAGTAATTGAAGGTTACCAAGAAATCATTCAT  
AATGAATCACAACGGTTCAAAAGCGTTAATGCAGATCAACCTCTTGAAAATGTT  
15 GTTGAAGACACGTATCAAACCTATCATCAAATATTTAGAAAAGATATGA

**FIGURE 134**

SEQ ID NO: 167

LRMNKMSAFITFEGPEGSGKTTVINEVYHRLVKDYDVIMTREPGGVPTGEEI  
5 RKIVLEGNDMDIRTEAMLFAASRREHLVLKVIPALKEGKVVLCDRYIDSSLAYQGY  
ARGIGVEEVRAALNEFAINGLYPDLTIYLNVS AEVGRERIIKNSRDQNRLDQEDLKFH  
EKVIEGYQEIIHNESQRFKSVNADQPLENVVEDTYQTIKYLEKI



**FIGURE 135**

SEQ ID NO: 168

TTGAGGATGAATAAAAATGTCAGCTTTTATAACTTTTGAGGGCCCAGAAG  
5 GCTCTGGAAAAACAACCTGTAATTAATGAAGTTTACCATAGATTAGTAAAAGATT  
ATGATGTCATTATGACTAGAGAACCAGGTGGTGTTCCTACTGGTGAAGAAATAC  
GTAAAATTGTATTAGAAGGCAATGATATGGACATTAGAACTGAAGCAATGTTA  
TTTGCTGCATCTAGAAGAGAACATCTTGTATTAAAGGTCATACCAGCTTTAAAA  
GAAGGTAAGGTTGTGTTGTGTGATCGCTATATCGATAGTTCATTAGCTTATCAA  
10 GGTATGCTAGAGGGATTGGCGTTGAAGAAGTAAGAGCATTAAACGAATTTGC  
AATAAATGGATTATATCCAGACTTGACGATTTATTTAAATGTTAGTGCTGAAGT  
AGGTCGCGAACGTATTATTAAAAATTCAAGAGATCAAAATAGATTAGATCAAG  
AAGATTTAAAGTTTCACGAAAAAGTAATTGAAGGTTACCAAGAAATCATTCAT  
AATGAATCACAACGGTTCAAAAGCGTTAATGCAGATCAACCTCTTGAAAATGTT  
15 GTTGAAGACACGTATCAAACCTATCATCAAATATTTAGAAAAGATATGA

**FIGURE 136**

SEQ ID NO: 169

LRMNKMSAFITFEGPEGSGKTTVINEVYHRLVKDYDVIMTREPGGVPTGEEI  
5 RKIVLEGNDMDIRTEAMLFAASRREHLVLKVIPALKEGKVVLCDRYIDSSLAYQGY  
ARGIGVEEVRAALNEFAINGLYPDLTIYLNVS AEVGRERIIKNSRDQNRLDQEDLKFH  
EKVIEGYQEIIHNESQRFKSVNADQPLENVVEDTYQTIKYLEKI

**FIGURE 137**

SEQ ID NO: 170

Forward PCR Primer

5 GCGGCGGCCCATATGAGTAAGGAGTTTATATAATG

SEQ ID NO: 171

10

Reverse PCR Primer

GCGCGGATCCTTATACTATTTCTTCATGGCTACTC

**FIGURE 138****TABLE 27 Properties of thymidylate kinase from *S. aureus***

TABLE 27 -- thymidylate kinase from <i>S. aureus</i> -- SEQ ID NO: 166-SEQ ID NO: 169	
Melting temperature (°C) of SEQ ID NO: 170 (forward PCR primer)	60
Restriction enzyme for SEQ ID NO: 170 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 171 (reverse PCR primer)	60
Restriction enzyme for SEQ ID NO: 171 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 166	633
Number of amino acid residues in SEQ ID NO: 167	210
Number of different nucleic acid residues between SEQ ID NO: 166 and SEQ ID NO: 168	0
Number of different amino acid residues between SEQ ID NO: 167 and SEQ ID NO: 169	0
Calculated molecular weight of SEQ ID NO: 167 polypeptide (kDa)	23.3
Calculated pI of SEQ ID NO: 167 polypeptide	4.9
Solubility of SEQ ID NO: 169 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching 100%
Amount of purified polypeptide having SEQ ID NO: 169, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	60.6
Amount of purified selmet labeled polypeptide having SEQ ID NO: 169, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	20.7
Amount of purified <sup>15</sup> N labeled polypeptide having SEQ ID NO: 169, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	17.95
Amount of purified polypeptide having SEQ ID NO: 169 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	121.2
Amount of purified selmet labeled polypeptide having SEQ ID NO: 169 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	51.2
Amount of purified <sup>15</sup> N labeled polypeptide having SEQ ID NO: 169 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	33.9
Z-score for the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 169, determined as described in EXAMPLE 9	3.7E-08

**FIGURE 138-B**

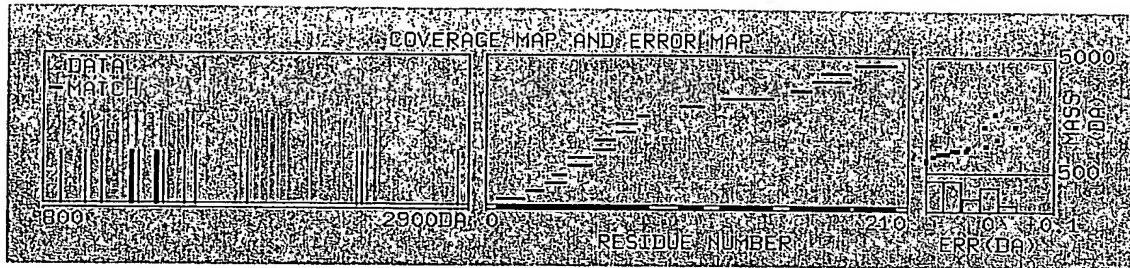
TABLE 27 -- thymidylate kinase from <i>S. aureus</i> -- SEQ ID NO: 166-SEQ ID NO: 169	
Number of matched peptides in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 169, determined as described in EXAMPLE 9	17
Minimum sequence coverage in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 169, determined as described in EXAMPLE 9	80
Calculated molecular weight of SEQ ID NO: 167 polypeptide (Da), determined as described in EXAMPLE 10	26116
Experimental molecular weight of SEQ ID NO: 169 polypeptide (Da), determined as described in EXAMPLE 10	26111
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. No interacting proteins were observed by using at least one of the methods described in those examples.	
Crystals of a polypeptide having the sequence of SEQ ID NO: 169, prepared and purified as described above and having a His tag, are obtained using the following conditions: 35% PEG400, HEPES pH 7.5, 0.2 M magnesium chloride. In addition, crystals of the same polypeptide may be prepared under the following conditions: 2M ammonium sulfate, 5% MPD. Further, crystals of the same polypeptide may be prepared under the following conditions: 35% PEG 400, sodium cacodylate pH 6.5, 0.2 M calcium acetate. Further, crystals of the same polypeptide may be prepared under the following conditions: 30% PEG 4000, TRIS-HCl pH 8.5, 0.2M lithium sulfate. Still further, crystals of the same polypeptide may be prepared under the following conditions: 2M ammonium sulfate, 2% PEG 400, HEPES pH 7.5. The crystals were prepared using the following method: 4°C, sitting drop, 15 mg polypeptide per ml of solution.	
Crystals of a selenomethionine-substituted polypeptide having the sequence of SEQ ID NO: 169, prepared and purified as described above and having a His tag, are obtained using the following conditions: 35% PEG400, HEPES pH 7.5, 0.2 M magnesium chloride. In addition, crystals of the same polypeptide may be prepared under the following conditions: 35% PEG400, sodium cacodylate pH 6.5, 0.2M calcium acetate. Further, crystals of the same polypeptide may be prepared under the following conditions: 2M ammonium sulfate, 2% PEG400, HEPES pH 7.5. Further, crystals of the same polypeptide may be prepared under the following conditions: 2M ammonium sulfate, sodium cacodylate pH 6.5, 0.2 M sodium chloride. Still further, crystals of the same polypeptide may be prepared under the following conditions: The crystals were prepared using the following method: 4°C, sitting drop, 15 mg polypeptide per ml of solution.	
Co-crystals of a polypeptide having the sequence of SEQ ID NO: 169 and 10 mM ADP, dTMP, or 2 mM ATP, are obtained using the following conditions: 1) for 10mM ADP, any of one of the three may be used: (a) PEG 400 30%, HEPES 0.1% pH 7.5 and magnesium chloride 0.2M, (b) ammonium sulfate 2M and HEPES 0.1M pH 7.5, or (c) PEG400 2%; 2) for 10 mM dTMP: ammonium sulfate 2.0M, 5% MPD; and 3) for 2 mM ATP: Ammonium sulfate 2.0M, 5% MPD. The concentration of the polypeptide in the solution used to prepare the crystal was 15mg/ml and the concentration of the ligand was as noted above. The crystals were prepared using the following method: 20°C, sitting drop. The subject crystallized polypeptide contains the His tag described above.	

**FIGURE 139****TABLE 28 Bioinformatic Analyses of thymidylate kinase from *S. aureus***

TABLE 28 -- thymidylate kinase from <i>S. aureus</i> -- SEQ ID NO: 166-SEQ ID NO: 169	
COG Category	Nucleotide transport and metabolism
COG ID Number	COG0125
Is SEQ ID NO: 167 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 167	Thymidylate Kinase, (Stmp_A)
Source organism for closest PDB protein to SEQ ID NO: 167	<i>Escherichia coli</i>
e-value for closest PDB Protein to SEQ ID NO: 167	1E-23
% Identity between SEQ ID NO: 167 and the closest protein from PDB	32
% Positives between SEQ ID NO: 167 and the closest protein from PDB	55
Number of Protein Hits in the VGDB to SEQ ID NO: 167	11
Number of Microorganisms having VGDB Hits to SEQ ID NO: 167	11
Microorganisms having VGDB Hits to SEQ ID NO: 167 <sup>1</sup>	[saur][bsub][efae][hinf][spne][paer][ecoli][nmen][ctra][rpxx][mgen]
First predicted epitopic region of SEQ ID NO: 167: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 172 :REHLVLKVIPAL, 1.208,76->87
Second predicted epitopic region of SEQ ID NO: 167: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 173 :EGKVVLCDRY, 1.19,89->98
Third predicted epitopic region of SEQ ID NO: 167: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 174 :INEVYHRLVKDYDVI, 1.155,24->38

- <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Escherichia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpxx* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

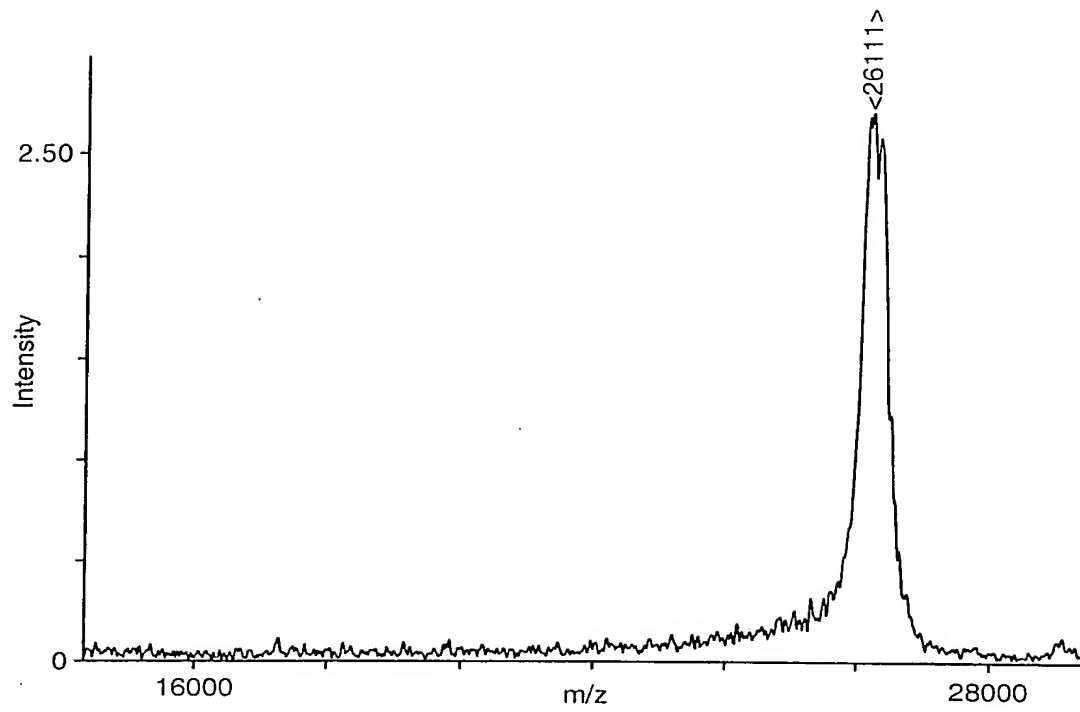
FIGURE 140



Note: click on the ☐ symbol to change column format.

Measured	Avg/	Computed	Error	<input type="checkbox"/> Residues		Missed	Peptide sequence
Mass (M)	Mono	Mass	(Da)	Start	To	Cut	
893.403	M	893.544	-0.141	76	82	1	REHLVLK
1011.327	M	1011.469	-0.142	34	41	0	DYDVIMTR
1095.413	M	1095.538	-0.124	66	75	0	TEAMLFAASR
1230.524	M	1230.635	-0.111	21	30	0	TTVINEVYHR
1239.498	M	1239.609	-0.110	42	53	0	EPGGVPTGEEIR
1251.543	M	1251.639	-0.096	66	76	1	TEAMLFAASRR
1273.542	M	1273.633	-0.091	55	65	0	IVLEGNDMDIR
1351.636	M	1351.716	-0.080	31	41	1	LVKDYDVIMTR
1367.631	M	1367.704	-0.072	42	54	1	EPGGVPTGEEIRK
1372.589	M	1372.658	-0.068	153	163	1	DQNRDQEDLK
1401.681	M	1401.728	-0.047	54	65	1	KIVLEGNDMDIR
1505.670	M	1505.714	-0.044	98	110	0	YIDSSLAYQGYAR
1556.711	M	1556.717	-0.006	6	20	0	MSAFITFEGPEGSRK
1813.911	M	1813.895	0.017	168	182	0	VIEGYQEIIHNSQR
2355.148	M	2355.159	-0.011	164	182	1	FHEKVIEGYQEIIHNSQR
2375.240	M	2375.184	0.056	185	205	0	SVNADQPLENVVEDTYQTIK
2851.490	M	2851.474	0.016	119	144	0	ALNEFAINGLYPDLTIYLNVSAEVGR

FIGURE 141





**FIGURE 142**

SEQ ID NO: 175

GTGTTTGATCAATTAGATATTGTAGAAGAAAGATACGAACAGTTAAATG  
5 AACTGTTAAGTGACCCAGATGTTGTAAATGATTCAGATAAATTACGTAAATATT  
CTAAAGAGCAAGCTGATTTACAAAAAAGTGTAGATGTTTATCGTAACTATAAA  
GCTAAAAAAGAAGAATTAGCTGATATTGAAGAAATGTTAAGTGAGACTGATGA  
TAAAGAAGAAGTAGAAATGTAAAAAGAGGAGAGTAATGGTATTAAAGCTGAA  
CTTCCAAATCTTGAAGAAGAGCTTAAAATATTATTGATTCCTAAAGATCCTAAT  
10 GATGACAAAGACGTTATTGTAGAAATAAGAGCAGCAGCAGGTGGTGATGAGGC  
TGCGATTTTTTGCTGGTGATTTAATGCGTATGTATTCAAAGTATGCTGAATCACA  
AGGATTCAAACTGAAATAGTAGAAGCGTCTGAAAGTGACCATGGTGGTTACA  
AAGAAATTAGTTTCTCAGTTTCTGGTAATGGCGCGTATAGTAAATTGAAATTTG  
AAAATGGTGCGCACCGCGTTCAACGTGTGCCTGAAACAGAATCAGGTGGACGT  
15 ATTCATACTTCAACAGCTACAGTGGCAGTTTTACCAGAAGTTGAAGATGTAGAA  
ATTGAAATTAGAAATGAAGATTTAAAAATCGACACGTATCGTTCAAGTGGTG  
AGGTGGTCAGCACGTAAACACAAGTACTCTGCAGTACGTATTACCCATTTACC  
AACTGGTGTCATTGCAACATCTTCTGAGAAGTCTCAAATTCAAAACCGTGAAAA  
AGCAATGAAAGTGTTAAAAGCACGTTTATACGATATGAAAGTTCAAGAAGAAC  
20 AACAAAAGTATGCGTCACAACGTAAATCAGCAGTCGGTACTGGTGATCGTTCA  
GAACGTATTCGAACTTATAATTATCCACAAAGCCGTGTAAACAGACCATTGTATA  
GGTCTAACGCTTCAAAAATTAGGGCAAATTATGGAAGGCCATTTAGAAGAAAT  
TATAGATGCACTGACTTTATCAGAGCAGACAGATAAATTGAAAGAAGTAAATA  
ATGGTGAATTATAA

**FIGURE 143**

SEQ ID NO: 176

VFDQLDIVEERYEQLNELLSDPDVVNDSDKLRKYSKEQADLQKTVDVYRN  
5 YKAKKEELADIEEMLSETDDKEEVEMMLKEESNGIKAELPNLEEELKILLIPKDPNDD  
KDVIVEIRAAAGGDEAAIFAGDLMRMYSKYAESQGFKTEIVEASESDHGGYKEISF  
SVSGNGAYSKLKFENGHRVQRPETESGGRIHTSTATVAVLPEVEDVEIEIRNEDL  
KIDTYRSSGAGGQHVNTTDSAVRITHLPTGVIATSSEKSQIQNREKAMKVLKARLY  
DMKVQEEQKQYASQRKSAVGTGDRSERIRTYNYPQSRVTDHRIGLTLQKLGQIME  
10 GHLEEIIDALTLEQTDKCLKELNNGEL

**FIGURE 144**

SEQ ID NO: 177

GTGTTTGATCAATTAGATATTGTAGAAGAAAGATACGAACAGTTAAATG  
5 AACTGTTAAGTGACCCAGATGTTGTAAATGATTCAGATAAATTACGTAAATATT  
CTAAAGAGCAAGCTGATTTACAAAAAACTGTAGATGTTTATCGTAACTATAAA  
GCTAAAAAAGAAGAATTAGCTGATATTGAAGAAATGTAAAGTGAGACTGATGA  
TAAAGAAGAAGTAGAAATGTAAAAAGAGGAGAGTAATGGTATTAAAGCTGAA  
CTTCCAAATCTTGAAGAAGAGCTTAAAATATTATTGATTCCTAAAGATCCTAAT  
10 GATGACAAAGACGTTATTGTAGAAATAAGAGCAGCAGCAGGTGGTGATGAGGC  
TGCGATTTTTTGCTGGTGATTTAATGCGTATGTATTCAAAGTATGCTGAATCACA  
AGGATTCAAACCTGAAATAGTAGAAGCGTCTGAAAGTGACCATGGTGGTTACA  
AAGAAATTAGTTTCTCAGTTTCTGGTAATGGCGCGTATAGTAAATTGAAATTTG  
AAAATGGTGCGCACCGCGTTCAACGTGTGCCTGAAACAGAATCAGGTGGACGT  
15 ATTCATACTTCAACAGCTACAGTGGCAGTTTTACCAGAAGTTGAAGATGTAGAA  
ATTGAAATTAGAAATGAAGATTTAAAAATCGACACGTATCGTTCAAGTGGTG  
AGGTGGTCAGCACGTAAACACAACCTGACTCTGCAGTACGTATTACCCATTTACC  
AACTGGTGTCATTGCAACATCTTCTGAGAAGTCTCAAATTCAAAACCGTGAAAA  
AGCAATGAAAGTGTTAAAAGCACGTTTATACGATATGAAAGTTCAAGAAGAAC  
20 AACAAAAGTATGCGTCACAACGTAAATCAGCAGTCGGTACTGGTGATCGTTCA  
GAACGTATTCGAACTTATAATTATCCACAAAGCCGTGTAAACAGACCATTGTATA  
GGTCTAACGCTTCAAAAATTAGGGCAAATTATGGAAGGCCATTTAGAAGAAAT  
TATAGATGCACTGACTTTATCAGAGCAGACAGATAAATTGAAAGAAGCTTAATA  
ATGGTGAATTATAA

**FIGURE 145**

SEQ ID NO: 178

VFDQLDIVEERYEQLNELLSDPDVVNDSDKLRKYSKEQADLQKTVDVYRN  
5 YKAKKEELADIEEMLSETDDKEEVEMMLKEESNGIKAELPNLEEELKILLIPKDPNDD  
KDVIVEIRAAAGGDEAAIFAGDLMRMYSKYAESQGFKTEIVEASESDHGGYKEISF  
SVSGNGAYSCLKFENGHRVQRPETESGGRIHTSTATVAVLPEVEDVEIEIRNEDL  
KIDTYRSSGAGGQHVNTTDSAVRITHLPTGVIATSSEKSQIQNREKAMKVLKARLY  
DMKVQEEQKQYASQRKSAVGTGDRSERIRTYNYPQSRVTDHCIGLTLQKLGQIME  
10 GHLEEIIDALTLSEQTDKCLKELNNGEL

**FIGURE 146**

SEQ ID NO: 179

Forward PCR Primer

5 GCGGCGGCCCATATGGCTGTA ACTAAGCTGGTTC

SEQ ID NO: 180

10

Reverse PCR Primer

GCGCGGATCCTTACCAGGATTTCTCAACGGGC

FIGURE 147

**TABLE 29 Properties of peptide chain release factor RF-1 from *S. aureus***

TABLE 29 -- peptide chain release factor RF-1 from <i>S. aureus</i> -- SEQ ID NO: 175-SEQ ID NO: 178	
Melting temperature (°C) of SEQ ID NO: 179 (forward PCR primer)	64
Restriction enzyme for SEQ ID NO: 179 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 180 (reverse PCR primer)	60
Restriction enzyme for SEQ ID NO: 180 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 175	1077
Number of amino acid residues in SEQ ID NO: 176	358
Number of different nucleic acid residues between SEQ ID NO: 175 and SEQ ID NO: 177	1
Number of different amino acid residues between SEQ ID NO: 176 and SEQ ID NO: 178	1
Calculated molecular weight of SEQ ID NO: 176 polypeptide (kDa)	39.7
Calculated pI of SEQ ID NO: 176 polypeptide	4.5
Solubility of SEQ ID NO: 178 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching 100%
Solubility of SEQ ID NO: 178 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	Approaching 100%
Amount of purified polypeptide having SEQ ID NO: 178, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	33.9
Amount of purified polypeptide having SEQ ID NO: 178 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	10.0
Z-score for the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 178, determined as described in EXAMPLE 9	7.7E-03
Number of matched peptides in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 178, determined as described in EXAMPLE 9	11
Minimum sequence coverage in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 178, determined as described in EXAMPLE 9	43
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. The identity of interacting proteins identified by using at least one of the methods described in those examples are: 50S ribosomal protein L7/L12 (gi 13700431), and 50S ribosomal protein L10 (gi 13700430).	

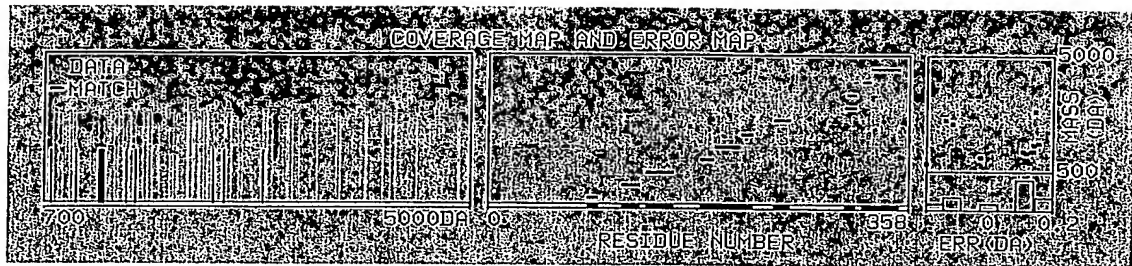
## FIGURE 148

**TABLE 30 Bioinformatic Analyses of peptide chain release factor RF-1 from *S. aureus***

TABLE 30 -- peptide chain release factor RF-1 from <i>S. aureus</i> -- SEQ ID NO: 175-SEQ ID NO: 178	
COG Category	Translation, ribosomal structure, and biogenesis
COG ID Number	COG0216
Is SEQ ID NO: 176 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 176	Release Factor 2 (1gqe)
Source organism for closest PDB protein to SEQ ID NO: 176	<i>Escherichia coli</i>
e-value for closest PDB Protein to SEQ ID NO: 176	3E-62
% Identity between SEQ ID NO: 176 and the closest protein from PDB	39
% Positives between SEQ ID NO: 176 and the closest protein from PDB	60
Number of Protein Hits in the VGDB to SEQ ID NO: 176	25
Number of Microorganisms having VGDB Hits to SEQ ID NO: 176	13
Microorganisms having VGDB Hits to SEQ ID NO: 176 <sup>1</sup>	[paer][hinf][ecoli][nmen][bsub][saur][spne][ctra][hpyl][bbur][rpxx][mgen][efae]
First predicted epitopic region of SEQ ID NO: 176: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 181 :TSTATVAVLPEVEDVEIE, 1.197,197->214
Second predicted epitopic region of SEQ ID NO: 176: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 182 :ELKILLIPK, 1.15,94->102
Third predicted epitopic region of SEQ ID NO: 176: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 183 :MKVLKAR, 1.13,268->274

- 5           <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Escherichia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpxx* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

FIGURE 149



Note: click on the ☐ symbol to change column format.

Measured Mass (M)	Avg/ Mono	Computed Mass	Error <input type="checkbox"/> (Da)	<input type="checkbox"/> Residues	Missed	Peptide sequence
				Start	To	
771.287	M	771.485	-0.198	319	325	0 IGLTLQK
1027.448	M	1027.472	-0.024	306	313	0 TYNYPQSR
1265.743	M	1265.625	0.118	217	226	1 NEDLKIDTYR
1283.801	M	1283.660	0.141	86	96	0 AELPNLEEELK
1296.765	M	1296.657	0.108	304	313	1 IRTYNYPQSR
1313.801	M	1313.668	0.133	183	194	1 VQRVPETESGGR
1552.985	M	1552.845	0.140	244	258	0 ITHLPTGVIATSSEK
1634.929	M	1634.771	0.158	116	132	0 AAAGGDEAAIFAGDLMR
2420.169	M	2420.279	-0.110	195	216	0 IHTSTATVAVLPEVEDVEIR
2531.011	M	2531.144	-0.133	137	159	1 YAESQGFKTEIVEASESDHGGYK
2923.663	M	2923.520	0.143	326	351	1 LGQIMEGHLEEIIDALTLSEQTDKLLK



**FIGURE 150**

SEQ ID NO: 184

ATGAAATTACAAAAACCAAAGGAACGCAGGATATTTTACCTGCTGAGT  
5 CTGCTAAGTGGCAGTACGTTGAGGGCTTTGCCCCGTGAGATTTTCAAACGCTACA  
ACTATGCAGAAGTGCGCACGCCTATTTTTGAGCATTACGAGGTTATCAGTCGCT  
CTGTCGGAGATACAACGGATATCGTAACCAAGGAAATGTACGATTTTTATGAC  
AAGGGTGACCGTCATATTACCCTCCGTCCAGAAGGAACTGCACCCGTTGTCCGT  
TCCTATGTGGAAAATAAACTTTTCGCCCCAGAAGTGCAAAAGCCAAGCAAGTT  
10 CTACTACATGGGACCTATGTTCCGTTATGAGCGTCCACAGGCAGGGCGCTTGCG  
CCAATTCCACCAGATTGGTGTTGAGTGTTTTGGCTCTAGCAATCCAGCTACCGA  
TGTGGAAACAATCGCTATGGCAGCCCATTTTTGAAGGAAATCGGTATTCAAGG  
TGTCAAATTGCACCTCAACACTCTTGGAATCCTGAGAGCCGTGCAGCCTACCG  
CCAAGCCTTGATTGACTATTTGACACCGCTCAAGGAGACCTTGTCTAAGGATAG  
15 CCAACGTCGCTTGAGAGGAAAATCCTCTTCGTGTCTTGGA CTCTAAGGAAAAAGA  
AGACAAGGTGGCAGTAGAGAATGCGCCGTCTATCTTGGA CTCTTCTTGATGAAG  
AAAGCCAAGCTCATTTTGATGCTGTGCGTCAGATGTTGGAAAATCTTGGA GTAG  
ATTACATCATCGATACCAATATGGTGCGTGGTCTGGA CTACTACAACCACACCA  
TTTTCGAGTTTATCACAGAGATTGAGGGCAATGACCTGACCGTCTGTGCGGGTG  
20 GTCGCTACGATGGTTTGGTTGCTTACTTTGGAGGCCCTGAAACTGCTGGATTTG  
GTTTTGGA CTTGGTGTAGAGCGCCTGCTTCTCATCCTTGAAAAGCAAGGTGTGA  
CCCTCCCTATCGAAAACGCCCTAGATGTCTATATCGCAGTCTTGGGCGAAGGGG  
CAAATATCAAGGCCTTGGAATTGGTACAGGCTCTTCGCCAACAAGGTTTCAAAG  
CAGAGCGTGATTACCTCAACCGTAAACTAAAAGCTCAGTTCAAGTCAGCCGAT  
25 GTCTTTGCGGCTAAGACCCTCATCACCCTAGGAGAGAGCGAAGTCGAAAGCGG  
ACAAGTGACGGTCAAGAACAACCAAACCCGAGAAGAAGTGCAAGTGTCAC TTG  
AGACAATCAGCCAAA ACTTCTCAGAAATCTTTGAAAACTAGGATTTTATACTC  
AATAA

**FIGURE 151**

SEQ ID NO: 185

          MKLQKPKGTQDILPAESAKWQYVEGFAREIFKRYNYAEVRTPIFEHYEVISR  
5  SVGDTTDIVTKEMYDFYDKGDRHITLRPEGTAPVVRSYVENKLFAPEVQKPSKFYY  
      MGPMFRYERPQAGRLRQFHQIGVECFGSSNPATDVETIAMAAHFLKEIGIGVKLH  
      LNTLGNPESRAAYRQALIDYLTPLKETLSKDSQRRLEENPLRVLDSKEKEDKVAVE  
      NAPSILDFLDEESQAHFDAVRQMLENLGVDYIIDTNMVRGLDYYNHTIFEITEIEG  
      NDLTVCAGGRYDGLVAYFGGPETAGFGFGLGVERLLLILEKQGVTLPIENALDVYI  
10  AVLGEGANIKALELVQALRQQGFKAERDYLNRLKKAQFKSADVFAAKTLITLGES  
      EVESGQVTVKNNQTREEVQVSLETISQNFSEIFEKLGFTYQ

**FIGURE 152**

SEQ ID NO: 186

ATGAAATTACAAAAACCAAAAGGAACGCAGGATATTTTACCTGCTGAGT  
5 CTGCTAAGTGGCAGTACGTTGAGGGCTTTGCCCGTGAAATTTTCAAGCGCTACA  
ACTATGCAGAAGTGCGCACGCCTATTTTTGAGCATTACGAGGTTATCAGTCGCT  
CTGTCGGAGATACAACGGATATCGTAACCAAGGAAATGTACGATTTTTTATGAC  
AAGGGTGACCGTCATATTACCCTCCGTCCAGAAGGAACTGCGCCCGTTGTCCGT  
TCCTATGTGGAAAATAAACTCTTCGCCCCAGAAGTGCAAAAGCCAAGCAAGTT  
10 CTACTATATGGGACCTATGTTCCGTTATGAGCGTCCACAGGCAGGGCGCTTGCG  
CCAATTCCACCAGATTGGTGTTGAGTGTTTTGGCTCTAGCAATCCAGCTACCGA  
TGTGGAAACAATCGTTATGGCAGCCCATTTTTTTGAAGGAAATCGGTATTCAAGG  
TGTCAAATTGCACCTCAACACTCTTGGAATCCTGAGAGCCGTGCAGCCTACCG  
CCAAGCCTTGATTGACTATTTGACACCGCTCAAGGAGACCTTGTCTAAGGATAG  
15 CCAACGTCGCTTGAGAGGAAAATCCTCTTCGTGTCTTGGA CTCTAAGGAAAAAGA  
AGACAAGGTGGCTGTAGAGAATGCGCCATCTATCTTGGATTTCCTTGATGAAGA  
AAGTCAAGCTCATTTTGATGCTGTGCGTCAGATGTTGGAAAATCTTGGAGTAGA  
CTACATCATCGATACCAATATGGTGCGTGGTCTGGACTACTACAACCACACCAT  
TTTCGAGTTTATCACAGAGATTGAGGGCAATGACTTGACAATCTGTGCGGGTGG  
20 TCGCTATGATGGTTTGGTTGCTTACTTTGGAGGCCCTGAAACTGCTGGATTGTTGGT  
TTTGGGCTTGGTGTAGAGCGCCTGCTTCTCATCCTTGAAAAACAAGGCGTGGCC  
CTCCCTATCGAAAACGCCCTAGATGTCTATATCGCAGTCTTGGGTGATGGAGCA  
AATGTCAAAGCCCTAGAACTAGTCCAAGTCCTTCGCCAACAAGGTTTCAAAGC  
AGAGCGTGATTACCTCAACCGTAAGCTCAAAGCTCAGTTCAAGTCAGCCGATGT  
25 CTTTGCGGCTAAGACCCTCATCACCCTAGGAGAGAGCGAAGTCGAAAGCGGGC  
AAGTGACGGTCAAGAACAACCAAAACCCGAGAAGAAGTGCAAGTGTCACCTGAG  
ACAATCAGCCAAA ACTTCTCAGAAATCTTTGAAAAACTAGGATTTTATACTCAA  
TAA

**FIGURE 153**

SEQ ID NO: 187

          MKLQKPKGTQDILPAESAKWQYVEGFAREIFKRYNYAEVRTPIFEHYEVISR  
5  SVGDTTDIVTKEMYDFYDKGDRHITLRPEGTAPVVRSYVENKLFAPEVQKPSKFYY  
      MGPMFRYERPQAGRLRQFHQIGVECFGSSNPATDVETIVMAAHFLKEIGIQGVKLH  
      LNTLGNPESRAAYRQALIDYLTPLKETLSKDSQRRLEENPLRVLDSKEKEDKVAVE  
      NAPSILDFLDEESQAHFDAVRQMLENLGVVDYIIDTNMVRGLDYYNHTIFEITEIEG  
      NDLTICAGGRYDGLVAYFGGPETAGFGFGLGVERLLLILEKQGVALPIENALDVYI  
10  AVLGDGANVKALELVQVLRQQGFKAERDYLNRKLKAQFKSADVFAAKTLITLGES  
      EVESGQVTVKNNQTREEVQVSLETISQNFSEIFEKLGFYTQ

**FIGURE 154**

SEQ ID NO: 188

Forward PCR Primer

5 GCGGCGGCCCATATGAAATTACAAAAACCAAAGG

SEQ ID NO: 189

10 GCGCGGATCCTTGAGTATAAAATCCTAGTTTTTC

**FIGURE 155****TABLE 31 Properties of histidine tRNA synthetase from *S. pneumoniae***

TABLE 31 -- histidine tRNA synthetase from <i>S. pneumoniae</i> -- SEQ ID NO: 184-SEQ ID NO: 187	
Melting temperature (°C) of SEQ ID NO: 188 (forward PCR primer)	58
Restriction enzyme for SEQ ID NO: 188 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 189 (reverse PCR primer)	60
Restriction enzyme for SEQ ID NO: 189 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 184	1290
Number of amino acid residues in SEQ ID NO: 185	429
Number of different nucleic acid residues between SEQ ID NO: 184 and SEQ ID NO: 186	36
Number of different amino acid residues between SEQ ID NO: 185 and SEQ ID NO: 187	6
Calculated molecular weight of SEQ ID NO: 185 polypeptide (kDa)	48.7
Calculated pI of SEQ ID NO: 185 polypeptide	4.9
Solubility of SEQ ID NO: 187 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approximately two thirds
Solubility of SEQ ID NO: 187 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	Approaching one third
Amount of purified polypeptide having SEQ ID NO: 187, prepared and purified as described in the Exemplification (mg/mL of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 3 at the C-terminus as described in EXAMPLE 6.	22.7
Amount of purified selmet labeled polypeptide having SEQ ID NO: 187, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 3 at the C-terminus as described in EXAMPLE 6.	1.3
Amount of purified polypeptide having SEQ ID NO: 187 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	70.8
Amount of purified selmet labeled polypeptide having SEQ ID NO: 187 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	6.2

**FIGURE 155-B**

TABLE 31 -- histidine tRNA synthetase from <i>S. pneumoniae</i> -- SEQ ID NO: 184-SEQ ID NO: 187	
Z-score for the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 187, determined as described in EXAMPLE 9	3.0E-06
Number of matched peptides in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 187, determined as described in EXAMPLE 9	19
Minimum sequence coverage in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 187, determined as described in EXAMPLE 9	47
Calculated molecular weight of SEQ ID NO: 185 polypeptide (Da), determined as described in EXAMPLE 10	50800
Experimental molecular weight of SEQ ID NO: 187 polypeptide (Da), determined as described in EXAMPLE 10	50223
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. The identity of an interacting protein identified by using at least one of the methods described in those examples is: 50kDa unidentified protein.	
Crystals of a polypeptide having the sequence of SEQ ID NO: 187, prepared and purified as described above and having a His tag, are obtained using the following conditions: ammonium sulfate 2M , TRIS 0.1M pH 8.5. In addition, crystals of the same polypeptide may be prepared under the following conditions: ammonium sulfate 2M , HEPES 0.1M pH 7.5 , PEG400 2%. Further, crystals of the same polypeptide may be prepared under the following conditions: 30% PEG 4000, sodium cacodylate pH 6.5, 0.2M sodium acetate. The crystals were prepared using the following method: 20°C, sitting drop, 15 mg polypeptide per ml of solution.	

## FIGURE 156

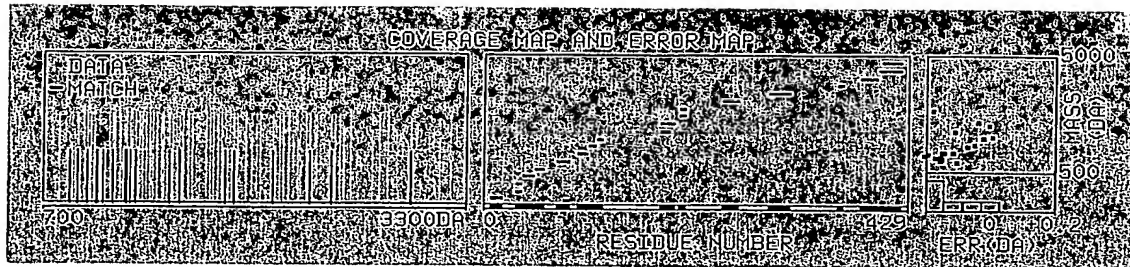
TABLE 32 Bioinformatic Analyses of histidine tRNA synthetase from *S. pneumoniae*

TABLE 32 -- histidine tRNA synthetase from <i>S. pneumoniae</i> -- SEQ ID NO: 184-SEQ ID NO: 187	
COG Category	Translation, ribosomal structure, and biogenesis
COG ID Number	COG0124
Is SEQ ID NO: 185 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 185	Histidyl-tRNA Synthetase (1qe0)
Source organism for closest PDB protein to SEQ ID NO: 185	<i>Staphylococcus aureus</i>
e-value for closest PDB Protein to SEQ ID NO: 185	1.0E-11
% Identity between SEQ ID NO: 185 and the closest protein from PDB	47
% Positives between SEQ ID NO: 185 and the closest protein from PDB	65
Number of Protein Hits in the VGDB to SEQ ID NO: 185	11
Number of Microorganisms having VGDB Hits to SEQ ID NO: 185	11
Microorganisms having VGDB Hits to SEQ ID NO: 185 <sup>1</sup>	[spne][bsub][hinf][ecoli][efae][paer][saur][nmen][ctra][rpxx][mgen]
First predicted epitopic region of SEQ ID NO: 185: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 190 :GLGVERLLLILEKQGV-TLPIENALDVYIAVLGE, 1.181,306->338
Second predicted epitopic region of SEQ ID NO: 185: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 191 :TAPVVRSY, 1.180,83->90
Third predicted epitopic region of SEQ ID NO: 185: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 192 :IKALELVQALRQQ, 1.152,342->354

- <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Eschericia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpxx* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.



FIGURE 157



Note: click on the ☐ symbol to change column format.

Measured	Avg/	Computed	Error	<input type="checkbox"/> Residues	Missed		
Mass (M)	Mono	Mass	(Da)	Start	To	Cut	Peptide sequence
869.377	M	869.460	-0.083	200	206	0	LEENPLR
913.304	M	913.429	-0.125	34	40	0	YNYAEVR
975.366	M	975.488	-0.122	115	122	0	YERPOAGR
1025.428	M	1025.561	-0.133	199	206	1	RLEENPLR
1035.372	M	1035.509	-0.137	358	365	1	AERDYLNR
1069.407	M	1069.530	-0.124	33	40	1	RNYAEVR
1134.413	M	1134.576	-0.164	53	63	0	SVGDTTIDIVTK
1210.373	M	1210.530	-0.156	106	114	0	FYYMGPMFR
1228.507	M	1228.629	-0.122	8	19	0	GTQDILPAESAK
1242.603	M	1242.697	-0.094	95	105	0	LFAPVQKPSK
1273.609	M	1273.727	-0.118	179	189	0	QALIDYLTPLK
1489.699	M	1489.756	-0.057	41	52	0	TPIFEHYEIVSR
1544.838	M	1544.878	-0.040	75	88	0	HITLRPEGTAPVVR
1832.011	M	1832.028	-0.017	179	194	1	QALIDYLTPLKETLSK
1889.005	M	1888.998	0.007	381	398	0	TLITLGESEVESGQVTVK
2122.950	M	2123.038	-0.088	242	259	0	QMLENLGVDYIIDTNMVR
2355.144	M	2355.147	-0.003	404	423	0	EEVQVSLETISQNFSEIFEK
2478.156	M	2478.184	-0.028	288	311	0	YDGLVAYFGGPETAGFGFGLGVER

**FIGURE 158**

SEQ ID NO: 193

ATGAAATCCTACCAAGCTGTCTACCAAATCCTATCTAAAGAAACCGACT  
5 ATATCAGCGGAGAAAAAATCGCAGAAAAACTATCCCTAAGCCGAACAGCAATT  
TGGAAAGCCATCAAGCGACTAGAACAAGAAGGCATTGAAATTGATAGTATCAA  
AAATAGAGGATATAAACTGATGAATGGTGACCTTATTCTTCCAGAGATTCTAGA  
AGAAAATCTTCCAATTAAAGTCAGCTTTAAACCCGAAACAAAATCAACACAAC  
TAGATGCAAAAAGAAGCAATTGATTTAGGCCATGAAGCAAATACCCTCTATCTA  
10 GCTTCCTATCAAACAGCAGGCCGAGGCCGTTTCAACGTTTCCTTCTACTCACCA  
CAAGGTGGTATTTATATGACACTCCATCTTAAACCAAATCTCCCCTATGACAAA  
TTACCATCCTACACACTACTTGTAGCTGGAGCTGTCTACAAAGCCATTAAGAAC  
CTAACTTTAATAGATGTCGACATAAAATGGGTCAATGATATCTATCTAAACAAT  
CATAAAATTGGAGGAATCCTTACTGAAGCAATGACCTCTGTAGAAACTGGCTTA  
15 GTCACAGATATCATTATTGGAGTAGGTATCAATTTCACTATTAAAGACTTCCCT  
CAGGAATTAAAAGAAAAAGCTGCCAGCTTATTTAAAGCTACAGCTCCTATAAC  
AAGGAATGAATTGATCATAGAAATCTGGCGTGCTTTCTTCGAAACACCAGCAG  
AAGAGCTATTATACCTATACAAAAAACAGTCATTCTAGGAAAAGAAGTC  
ACTTTCACACTAGAGCAAAAAGACTACAAGGGACTTGCTAAAGACATCTCAGA  
20 AAATGGAAAACTTTTAGTTCAATGTGATAACGGAAAAGAAATCTGGCTAAATA  
GTGGCGAAATTTCTCTCAATAGTTGGAAGTAA

**FIGURE 159**

SEQ ID NO: 194

MKSYQAVYQILSKETDYISGEKIAEKLSLSRTAIWKAIKRLEQEGIEIDSIKNR  
5 GYKLMNGDLILPEILEENLPIKVSFKPETKSTQLDAKEAIDLGHEANTLYLASQTA  
GRGRFQRSFYSPQGGIYMTLHLKPNLPYDKLPSYTLLVAGAVYKAIKNLTLIDVDIK  
WVNDIYLNHKGIGILTEAMTSVETGLVTDIIIGVGINFTIKDFPQELKEKAASLFKA  
TAPITRNELIIEIWRAFFETPAEELLYLYKKQSFILGKEVTFTLEQKDYKGLAKDISEN  
GKLLVQCDNGKEIWLNSGEISLNSWK

**FIGURE 160**

SEQ ID NO: 195

ATGAAATCCTACCAAGCTGTCTACCAAATCCTATCTAAAGAAACCGACT  
5 ATATCAGCGGAGAAAAAATCGCAGAAAAACTATCCCTAAGCCGAACAGCAATT  
TGGAAAGCCATCAAGCGACTAGAACAAGAAGGCATTGAAATTGATAGTATCAA  
AAATAGAGGATATAAACTGATGAATGGTGACCTTATTCTTCCAGAGATTCTAGA  
AGAAAATCTTCCAATTAAAGTCAGCTTTAAACCCGAAACAAAATCAACACAAC  
TAGATGCAAAAGAAGCAATTGATTTAGGCCATGAAGCAAATACCCTCTATCTA  
10 GCTTCCTATCAAACAGCAGGCCGAGGCCGTTTTCAACGTTTCCTTCTACTCACCA  
CAAGGTGGTATTTATATGACACTCCATCTTAAACCAAATCTCCCCTATGACAAA  
TTACCATCCTACACACTACTTGTAGCTGGAGCTGTCTACAAAGCCATTAAGAAC  
CTAACTTTAATAGATGTCGACATAAAATGGGTCAATGATATCTATCTAAACAAT  
CATAAAATTGGAGGAATCCTTACTGAAGCAATGACCTCTGTAGAAACTGGCTTA  
15 GTCACAGATATCATTATTGGAGTAGGTATCAATTTCACTATTAAAGACTTCCCT  
CAGGAATTAAAAGAAAAAGCTGCCAGCTTATTTAAAGCTACAGCTCCTATAAC  
AAGGAATGAATTGATCATAGAAATCTGGCGTACTTTCTTCGAAACACCAGCAG  
AAGAGCTATTATACCTATACAAAAAACAGTCATTTCATTCTAGGAAAAGAAGTC  
ACTTTCACACTAGAGCAAAAAGACTACAAGGGACTTGCTAAAGACATCTCAGA  
20 AAATGGAAAACCTTTTAGTTCAATGTGATAACGGAAAAGAAATCTGGCTAAATA  
GTGGCGAAATTTCTCTCAATAGTTGGAAGTAA

**FIGURE 161**

SEQ ID NO: 196

MKSYQAVYQILSKETDYISGEKIAEKLSLSRTAIWKAIKRLEQEGIEDSIKNR  
5 GYKLMNGDLILPEILEENLPIKVSFKPETKSTQLDAKEAIDLGHEANTLYLASQTA  
GRGRFQRSFYSPQGGIYMTLHLKPNLPYDKLPSYTLLVAGAVYKAIKNLTLIDVDIK  
WVNDIYLNHNKIGGILTEAMTSVETGLVTDIIIGVGINFTIKDFPQELKEKAASLFKA  
TAPITRNELIIEIWRTFFETPAEELLYLYKKQSFILGKEVTFTLEQKDYKGLAKDISEN  
GKLLVQCDNGKEIWLNSGEISLNSWK

10

**FIGURE 162**

SEQ ID NO: 197

Forward PCR Primer

5 GCGGCGGCCCATATGAAATCCTACCAAGCTGTC

SEQ ID NO: 198

10

Reverse PCR Primer

GCGCGGATCCCTTCCAACCTATTGAGAGAAATTTC

FIGURE 163

TABLE 33 Properties of BirA bifunctional protein from *S. pneumoniae*

TABLE 33 -- BirA bifunctional protein from <i>S. pneumoniae</i> -- SEQ ID NO: 193-SEQ ID NO: 196	
Melting temperature (°C) of SEQ ID NO: 197 (forward PCR primer)	60
Restriction enzyme for SEQ ID NO: 197 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 198 (reverse PCR primer)	64
Restriction enzyme for SEQ ID NO: 198 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 193	936
Number of amino acid residues in SEQ ID NO: 194	311
Number of different nucleic acid residues between SEQ ID NO: 193 and SEQ ID NO: 195	1
Number of different amino acid residues between SEQ ID NO: 194 and SEQ ID NO: 196	1
Calculated molecular weight of SEQ ID NO: 194 polypeptide (kDa)	35.2
Calculated pI of SEQ ID NO: 194 polypeptide	7.2
Solubility of SEQ ID NO: 196 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one third
Solubility of SEQ ID NO: 196 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	Approaching one third
Amount of purified polypeptide having SEQ ID NO: 196, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified has the additional amino acid residues from the removed His tag at the N-terminus as described in EXAMPLE 6.	4.2
Amount of purified selmet labeled polypeptide having SEQ ID NO: 196, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	2.7
Amount of purified polypeptide having SEQ ID NO: 196 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	6.5
Amount of purified selmet labeled polypeptide having SEQ ID NO: 196 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	2.0

**FIGURE 163-B**

TABLE 33 -- BirA bifunctional protein from <i>S. pneumoniae</i> -- SEQ ID NO: 193-SEQ ID NO: 196	
Z-score for the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 196, determined as described in EXAMPLE 9	4.8E-05
Number of matched peptides in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 196, determined as described in EXAMPLE 9	18
Minimum sequence coverage in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 196, determined as described in EXAMPLE 9	54
Calculated molecular weight of SEQ ID NO: 194 polypeptide (Da), determined as described in EXAMPLE 10	37258
Experimental molecular weight of SEQ ID NO: 196 polypeptide (Da), determined as described in EXAMPLE 10	37461
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. The identity of an interacting protein identified by using at least one of the methods described in those examples is: 32kDa unidentified protein.	
Crystals of a selenomethionine-substituted polypeptide having the sequence of SEQ ID NO: 196, prepared and purified as described above and having a His tag, are obtained using the following conditions: PEG400 35%, Na cacodylate 0.1M, pH 6.5, 0.2M calcium acetate. The crystals were prepared using the following method: 20°C, sitting drop, 2 mg polypeptide per ml of solution.	



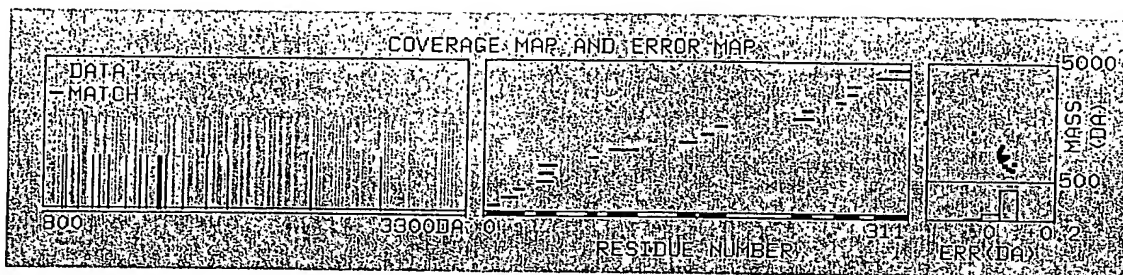
FIGURE 164

TABLE 34 Bioinformatic Analyses of BirA bifunctional protein from *S. pneumoniae*

TABLE 34 -- BirA bifunctional protein from <i>S. pneumoniae</i> -- SEQ ID NO: 193-SEQ ID NO: 196	
COG Category	Transcription
COG ID Number	COG1654
Is SEQ ID NO: 194 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 194	BirA Bifunctional Protein (1hxd)
Source organism for closest PDB protein to SEQ ID NO: 194	<i>Escherichia coli</i>
e-value for closest PDB Protein to SEQ ID NO: 194	8.0E-23
% Identity between SEQ ID NO: 194 and the closest protein from PDB	28
% Positives between SEQ ID NO: 194 and the closest protein from PDB	49
Number of Protein Hits in the VGDB to SEQ ID NO: 194	8
Number of Microorganisms having VGDB Hits to SEQ ID NO: 194	8
Microorganisms having VGDB Hits to SEQ ID NO: 194 <sup>1</sup>	[spne][efae][saur][bsub] [paer][ecoli][nmen][ctra]
First predicted epitopic region of SEQ ID NO: 194: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 199 :YMTLHLKPNLPYD-KLPSYTLVAGAVYKAIKNLTLIDVDIK. 1.181,128->168
Second predicted epitopic region of SEQ ID NO: 194: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 200 :KLLVQCD, 1.159,287->293
Third predicted epitopic region of SEQ ID NO: 194: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 201 :YQAVYQILS, 1.149,4->12

- <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Escherichia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpax* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

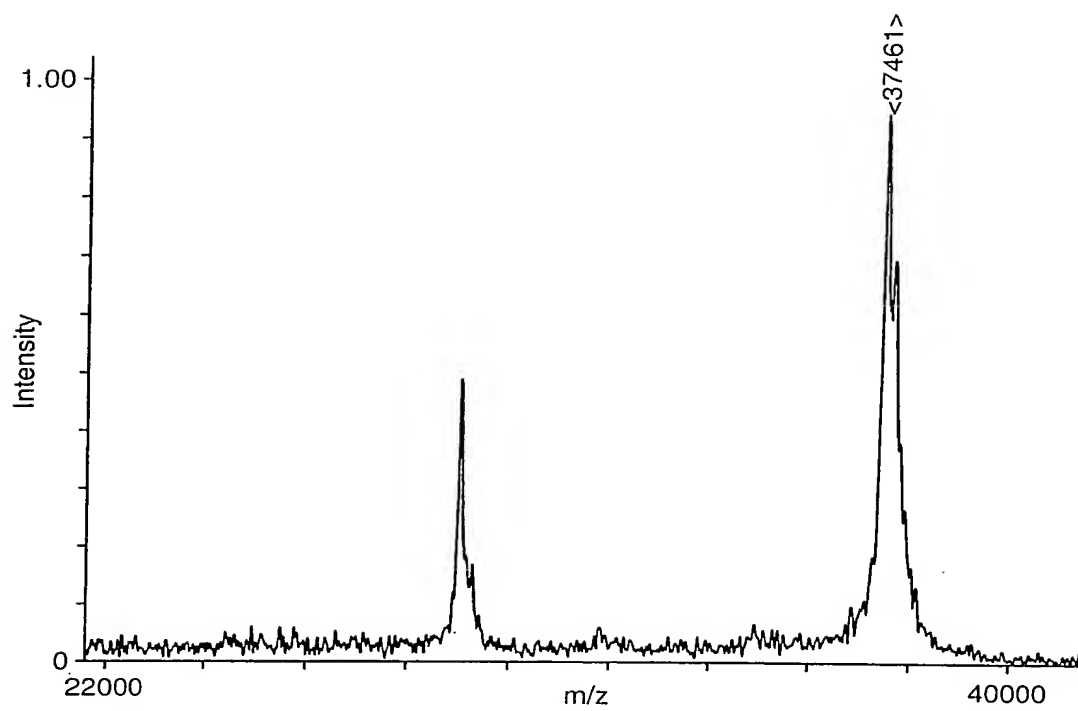
FIGURE 165



Note: click on the ☐ symbol to change column format.

Measured	Avg/	Computed	Error	<input type="checkbox"/> Residues	Missed	
Mass (M)	Mono	Mass	(Da)	Start	To	Cut Peptide sequence
919.616	M	919.549	0.067	257	264	1 KQSFILGK
934.582	M	934.512	0.070	77	84	0 VSFKPETK
1015.661	M	1015.602	0.059	23	31	1 IAEKLSLSR
1093.612	M	1093.565	0.047	265	273	0 EVTFTLEQK
1142.694	M	1142.654	0.040	159	168	0 NLTLIDVDIK
1184.727	M	1184.655	0.072	233	241	0 NELIIEIWR
1298.714	M	1298.687	0.027	3	13	0 SYQAVYQILSK
1372.746	M	1372.708	0.039	41	52	0 LEQEGIEIDSIK
1414.746	M	1414.699	0.047	169	179	0 WNDIYLNNHK
1481.760	M	1481.724	0.036	14	26	1 ETDYISGEKIAEK
1493.875	M	1493.849	0.027	142	155	0 LPSYTLVAGAVYK
1499.782	M	1499.750	0.032	265	276	1 EVTFTLEQKDYK
1528.861	M	1528.809	0.052	40	52	1 RLEQEGIEIDSIK
1642.877	M	1642.852	0.025	41	54	1 LEQEGIEIDSIKNR
1774.919	M	1774.888	0.031	297	311	0 EIWLNSGEISLNSWK
1895.095	M	1895.062	0.033	226	241	1 ATAPITRNELIIEIWR
2392.113	M	2392.165	-0.052	92	113	0 EAIDLGHEANTLYLASQTAGR

FIGURE 166



**FIGURE 167**

SEQ ID NO: 202

ATGGGATATACAGTTGCTGTAGTCGGCGCGACAGGTGCTGTCTGGTGCTC  
5 AGATGATAAAAATGTTGGAAGAATCAACACTTCCAATCGATAAAATTCGTTAC  
CTTGCTTCTGCACGTTCAAGCAGGTAAGTCATTGAAATTTAAAGATCAAGATATT  
ACAATTGAAGAAACGACTGAAACAGCTTTTGAAGGAGTTGATATTGCTCTCTTT  
TCAGCAGGTAGTTCTACATCAGCTAAGTATGCACCATACGCAGTAAAAGCTGG  
CGTGGTAGTAGTAGATAATACATCTTATTTCCGTCAAATCCAGATGTTCCCTTT  
10 GGTGTTCCAGAGGTCAATGCTCATGCACTTGATGCTCACAACGGAATCATTGC  
CTGCCCTAATTGTTCAACAATTCAAATGATGGTGGCTCTTGAGCCGGTTCGCCA  
AAAATGGGGCTTGGACCGTATCATTGTTTCAACTTATCAAGCCGTTTCAGGTGC  
TGGTATGGGAGCAATTCTTGAGACACAACGTGAACTTCGTGAAGTCTTGAATGA  
TGGTGTGAAACCACGTGATTTGCATGCGGAAATCTTGCCTTCAGGTGGTGACAA  
15 GAAACATTATCCTATCGCCTTTAACGCTCTTCCACAAATTGATGTTTTCACTGAT  
AATGATTACACGTACGAAGAGATGAAGATGACCAAGGAACTAAGAAAATTAT  
GGAAGATGATAGCATTGCAGTATCTGCAACATGTGTGCGTATTCCAGTCTTGTC  
AGCTCACTCTGAGTCTGTTTATATCGAAACAAAAGAAGTGGCTCCAATCGAAG  
AAGTAAAAGCAGCTATCGCAGCCTTCCCAGGTGCTGTTCTTGAAGATGATGTAG  
20 CTCATCAAATCTATCCTCAAGCTATCAATGCAGTTGGTTCGCGTGATACCTTTGT  
TGGTCGTATCCGTAAAGACTTGGATGCAGAAAAAGGAATTCACATGTGGGTTG  
TTTCAGATAACCTTCTCAAAGGTGCTGCTTGGAAGTCAAGTTCAGATTGCTGAAA  
CTCTTCATGAACGTGGATTGGTTCGTCCAACAGCCGAATTGAAATTTGAATTAA  
AATAG

**FIGURE 168**

SEQ ID NO: 203

MGYTVAVVGATGAVGAQMIKMLEESTLPIDKIRYLASARSAGKSLKFKDQ  
5 DITIEETTETA FEGVDIALFSAGSSTSAKYAPYAVKAGVVVDNTSYFRQNPDVPLV  
VPEVNAHALDAHNGIACPN CSTIQMMVALEPVRQKWGLDRIIVSTYQAVSGAGM  
GAILETQRELREVLNDGVKPRDLHAEILPSGGDKKHYP IAFNALPQIDVFTDNDYTY  
EEMKMTKETKKIMEDDSIAVSATCVRIPVLSAHSESVYIETKEVAPIEEVKAAIAAFP  
GAVLEDDVAHQIYPQAINAVGSRDTFVGRIRKDLDAEKGIHMWVVS DNLLKGAA  
10 WNSVQIAETLHERGLVRPTAELKFELK

**FIGURE 169**

SEQ ID NO: 204

ATGGGATATACAGTTGCTGTAGTCGGCGCGACAGGTGCTGTCTGGTGCTC  
5 AGATGATAAAAATGTTGGAAGAATCAACACTTCCAATTGATAAAAATCCGTTAC  
CTTGCTTCTGCACGTTCAAGCAGGTAAGTCATTGAAATTTAAAGATCAAGATATT  
ACGATTGAAGAAACGACTGAAACAGCTTTTGAAGGAGTTGATATTGCTCTCTTT  
TCAGCAGGTGATTTCGACATCAGCTAAGTATGCACCATACGCAGTAAAAGCTGG  
CGTGGTAGTAGTGGATAATACATCTTATTTCCGTCAAAATCCAGATGTTCCCTTT  
10 GGTGTTCCAGAGGTCAATGCTCATGCACTTGATGCCCACAACGGAATCATTGC  
CTGCCCTAACTGTTCAACAATCCAAATGATGGTGGCTCTTGAGCCGGTTCGCCA  
AAAATGGGGCTTGGACCGTATCATTGTTTCAACTTATCAAGCCGTTTCAGGTGC  
TGGTATGGGAGCAATTCTTGAGACACAACGTGAACTTCGTGAAGTCTTGAATGA  
TGGTGTGAAACCACGTGATTTGCATGCGGAAATCTTACCTTCAGGCGGTGACAA  
15 GAAACATTATCCTATCGCCTTCAATGCTCTTCCACAAATCGATGTCTTCACTGAC  
AATGATTACACTTACGAAGAGATGAAGATGACCAAGGAAACTAAGAAAATTAT  
GGAAGATGATAGCATTGCAGTATCTGCAACATGTGTACGTATTCCAGTCTTGTC  
AGCTCACTCTGAGTCTGTTTATATCGAAACAAAAGAAGTGGCTCCAATCGAAG  
AAGTAAAAGCAGCTATCGCAGCCTTCCCAGGTGCTGTTCTTGAAGATGATGTAG  
20 CTCATCAAATCTATCCTCAAGCTATCAATGCAGTTGGTTCGCGTGATACCTTTGT  
TGGTCGTATCCGTAAAGACTTGGATGCAGAAAAAGGAATTCACATGTGGGTTG  
TTTCAGATAACCTTCTCAAAGGTGCTGCTTGGAAGTCAAGTTCAGATTGCTGAAA  
CTCTTCATGAACGTGGATTGGTTCGTCCAACAGCCGAATTGAAATTTGAATTAA  
AATAG

**FIGURE 170**

SEQ ID NO: 205

MGYTVAVVGATGAVGAQMIKMLEESTLPIDKIRYLASARSAGKSLKFKDQ  
5 DITIEETTETAFEGVDIALFSAGDSTSAKYAPYAVKAGVVVDNTSYFRQNPDVPL  
VVPEVNAHALDAHNGIACPNCSTIQMMVALEPVRQKWGLDRIIVSTYQAVSGAG  
MGAILETQRELREVLNDGVKPRDLHAEILPSGGDKKHYPYAFNALPQIDVFTDNDY  
TYEEMKMTKETKKIMEDDSIAVSATCVRIPVLSAHSESVYIETKEVAPIEEVKAAIA  
AFPGAVLEDDVAHQIYPQAINAVGSRDTFVGRIRKDLDAEKGIHMWVVSNDLLKG  
10 AAWNSVQIAETLHERGLVRPTAELKFELK

**FIGURE 171**

SEQ ID NO: 206

Forward PCR Primer

5 GCGGCGGCCCATATGGGATATACAGTTGCTGTAG

SEQ ID NO: 207

10

Reverse PCR Primer

GCGCGGATCCTTTTAATTCAAATTTCAATTCGGC



FIGURE 172

**TABLE 35 Properties of putative PTS system enzyme II A component from *S. pneumoniae***

TABLE 35 -- putative PTS system enzyme II A component from <i>S. pneumoniae</i> -- SEQ ID NO: 202-SEQ ID NO: 205	
Melting temperature (°C) of SEQ ID NO: 206 (forward PCR primer)	62
Restriction enzyme for SEQ ID NO: 206 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 207 (reverse PCR primer)	60
Restriction enzyme for SEQ ID NO: 207 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 202	1077
Number of amino acid residues in SEQ ID NO: 203	358
Number of different nucleic acid residues between SEQ ID NO: 202 and SEQ ID NO: 204	19
Number of different amino acid residues between SEQ ID NO: 203 and SEQ ID NO: 205	1
Calculated molecular weight of SEQ ID NO: 203 polypeptide (kDa)	38.97
Calculated pI of SEQ ID NO: 203 polypeptide	4.9
Solubility of SEQ ID NO: 205 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one third
Solubility of SEQ ID NO: 205 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	Approximately two thirds
Amount of purified polypeptide having SEQ ID NO: 205, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	10.0
Amount of purified selmet labeled polypeptide having SEQ ID NO: 205, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	5.8
Amount of purified polypeptide having SEQ ID NO: 205 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	27.0
Amount of purified selmet labeled polypeptide having SEQ ID NO: 205 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	14.5

**FIGURE 172-B**

TABLE 35 -- putative PTS system enzyme II A component from <i>S. pneumoniae</i> -- SEQ ID NO: 202-SEQ ID NO: 205	
Calculated molecular weight of SEQ ID NO: 203 polypeptide (Da), determined as described in EXAMPLE 10	41007
Experimental molecular weight of SEQ ID NO: 205 polypeptide (Da), determined as described in EXAMPLE 10	41372
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. No interacting proteins were observed by using at least one of the methods described in those examples.	
Crystals of a polypeptide having the sequence of SEQ ID NO: 205, prepared and purified as described above and having a His tag, are obtained using the following conditions: 30% PEG 1500. In addition, crystals of the same polypeptide may be prepared under the following conditions: 30% PEG 4000, sodium citrate pH 5.5, 0.2 M ammonium acetate. Further, crystals of the same polypeptide may be prepared under the following conditions: 20% PEG 8000, sodium citrate pH 5.5, 0.2M magnesium chloride. The crystals were prepared using the following method: 20°C, sitting drop, 10 mg polypeptide per ml of solution.	
Crystals of a selenomethionine-substituted polypeptide having the sequence of SEQ ID NO: 205, prepared and purified as described above and having a His tag, are obtained using the following condition: 30% PEG 1500. The crystals were prepared using the following method: 20°C, sitting drop, 14.5 mg polypeptide per ml of solution.	

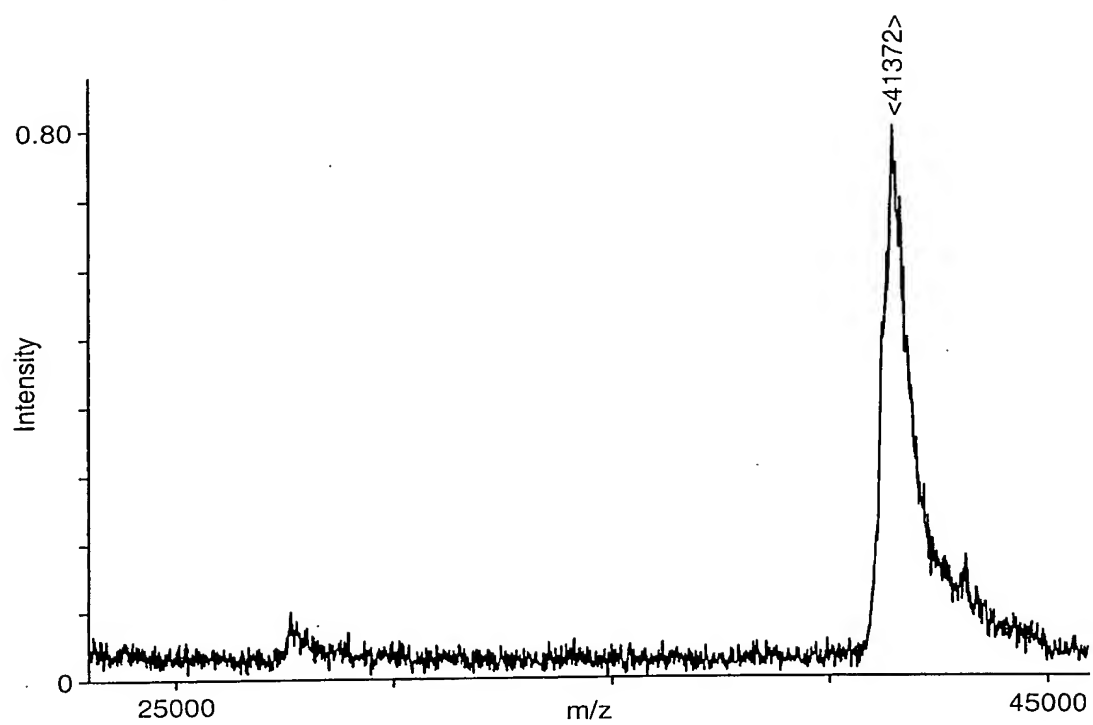
## FIGURE 173

**TABLE 36 Bioinformatic Analyses of putative PTS system enzyme II A component from *S. pneumoniae***

TABLE 36 -- putative PTS system enzyme II A component from <i>S. pneumoniae</i> -- SEQ ID NO: 202-SEQ ID NO: 205	
COG Category	Amino acid transport and metabolism
COG ID Number	COG0136
Is SEQ ID NO: 203 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 203	Aspartate-Semialdehyde Dehydrogenase (1gl3)
Source organism for closest PDB protein to SEQ ID NO: 203	<i>Escherichia coli</i>
e-value for closest PDB Protein to SEQ ID NO: 203	3.0E-16
% Identity between SEQ ID NO: 203 and the closest protein from PDB	26
% Positives between SEQ ID NO: 203 and the closest protein from PDB	43
Number of Protein Hits in the VGDB to SEQ ID NO: 203	8
Number of Microorganisms having VGDB Hits to SEQ ID NO: 203	8
Microorganisms having VGDB Hits to SEQ ID NO: 203 <sup>1</sup>	[spne][efae][bsub][hpyl] [rpxx][saur][ecoli][paer]
First predicted epitopic region of SEQ ID NO: 203: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 208 :SIAVSATCVRIPVLSAHSESV-YIETKEVAPIEEVKAAIAAFPGAVLEDDVAHQIYPQAINAV, 1.217,236->297
Second predicted epitopic region of SEQ ID NO: 203: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 209 :SAKYAPYAVKAGVVVDNT, 1.200,77->95
Third predicted epitopic region of SEQ ID NO: 203: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 210 :NPDVPLVVPEVNAHALD, 1.199,101->117

- 5           <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Escherichia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpxx* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

FIGURE 174



**FIGURE 175**

SEQ ID NO: 211

ATGGATTAAAGCAATACGTATCAGAAGTTCAAGATTGGCCGAAACCAG  
5 GTGTTAGTTTCAAGGATATTACTACAATTATGGATAATGGTGAAGCATATGGCT  
ATGCAACAGATAAAATTGTAGAATACGCAAAAGACAGAGATGTTGATATCGTT  
GTAGGACCTGAAGCGCGTGGCTTTATCATTGGCTGTCCTGTAGCTTATTCAATG  
GGGATTGGCTTTGCACCTGTTAGAAAAGAAGGGAAATTACCTCGTGAAGTCATT  
CGTTATGAGTATGACCTAGAATATGGTACAAATGTTTTAACAATGCACAAAGAT  
10 GCAATTAAACCAGGTCAACGTGTGTTAATTACAGATGATTTATTAGCTACTGGT  
GGTACGATTGAAGCAGCAATAAAATTAGTTGAAAAATTAGGCGGTATCGTAGT  
AGGTATTGCATTTATAATTGAATTGAAATATTTAAATGGTATTGAAAAAATTAA  
AGATTACGATGTTATGAGTTTAATCTCATACGACGAATAA

**FIGURE 176**

SEQ ID NO: 212

MDLKQYVSEVQDWPKPGVSFKDITTIMDNGEAYGYATDKIVEYAKDRDVD  
5 IVVGPEARGFIIIGCPVAYSMGIGFAPVRKEGKLPREVIRYEYDLEYGTNVLTMHKD  
AIKPGQRVLITDDLATGGTIEAAIKLVEKLGGIVVGIAFIELKYLNIEKIKDYDV  
MSLISYDE

**FIGURE 177**

SEQ ID NO: 213

```
      ATGGATTTAAAGCAATACGTATCAGAAGTTCAAGATTGGCCGAAACCAG
5  GTGTTAGTTTCAAGGATATTACTACAATTATGGATAATGGTGAAGCATATGGCT
  ATGCAACAGATAAAATTGTAGAATACGCAAAGACAGAGATGTTGATATCGTT
  GTAGGACCTGAAGCGCGTGGCTTTATCATTGGCTGTCCTGTAGCTTATTCAATG
  GGGATTGGCTTTGCACCTGTTAGAAAAGAAGGGAAATTACCTCGTGAAGTCATT
  CGTTATGAGTATGACCTAGAATATGGTACAAATGTTTTAACAATGCACAAAGAT
10 GCAATTAAACCAGGTCAACGTGTGTTAATTACAGATGATTTATTAGCTACTGGT
  GGTACGATTGAAGCAGCAATAAAATTAGTTGAAAAATTAGGCGGTATCGTAGT
  AGGTATTGCATTTATAATTGAATTGAAATATTTAAATGGTATTGAAAAAATTAA
  AGATTACGATGTTATGAGTTTAATCTCATACGACGAATAA
```

**FIGURE 178**

SEQ ID NO: 214

MDLKQYVSEVQDWPKPGVSFKDITTIMDNGEAYGYATDKIVEYAKDRDVD  
5 IVVGPEARGFIIIGCPVAYSMGIGFAPVRKEGKLPREVIRYEYDLEYGTNVLTMHKD  
AIKPGQRVLITDDLLATGGTIEAAIKLVEKLGGIVVGIAFIIELKYLNIEKIKDYDV  
MSLISYDE



**FIGURE 179**

SEQ ID NO: 215

Forward PCR Primer

5 GCGGCGGCATTAATATGGATTAAAGCAATACGTATC

SEQ ID NO: 216

10

Reverse PCR Primer

GCGCGGATCCTTCGTCGTATGAGATTAAACTC

**FIGURE 180****TABLE 36 Properties of adenine phosphoribosyltransferase from *S. aureus***

TABLE 36 -- adenine phosphoribosyltransferase from <i>S. aureus</i> -- SEQ ID NO: 211-SEQ ID NO: 214	
Melting temperature (°C) of SEQ ID NO: 215 (forward PCR primer)	60
Restriction enzyme for SEQ ID NO: 215 (forward PCR primer)	AseI
Melting temperature (°C) of SEQ ID NO: 216 (reverse PCR primer)	60
Restriction enzyme for SEQ ID NO: 216 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 211	519
Number of amino acid residues in SEQ ID NO: 212	172
Number of different nucleic acid residues between SEQ ID NO: 211 and SEQ ID NO: 213	0
Number of different amino acid residues between SEQ ID NO: 212 and SEQ ID NO: 214	0
Calculated molecular weight of SEQ ID NO: 212 polypeptide (kDa)	19.1
Calculated pI of SEQ ID NO: 212 polypeptide	4.5
Solubility of SEQ ID NO: 214 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching 100%
Amount of purified polypeptide having SEQ ID NO: 214, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	33.14
Amount of purified <sup>15</sup> N labeled polypeptide having SEQ ID NO: 214, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	16.6
Amount of purified polypeptide having SEQ ID NO: 214 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	66.28
Amount of purified <sup>15</sup> N labeled polypeptide having SEQ ID NO: 214 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	33.1

**FIGURE 180-B**

TABLE 36 -- adenine phosphoribosyltransferase from <i>S. aureus</i> -- SEQ ID NO: 211-SEQ ID NO: 214	
Z-score for the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 214, determined as described in EXAMPLE 9	9.7E-6
Number of matched peptides in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 214, determined as described in EXAMPLE 9	12
Minimum sequence coverage in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 214, determined as described in EXAMPLE 9	78
Calculated molecular weight of SEQ ID NO: 212 polypeptide (Da), determined as described in EXAMPLE 10	21148
Experimental molecular weight of SEQ ID NO: 214 polypeptide (Da), determined as described in EXAMPLE 10	21263
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. No interacting proteins were observed by using at least one of the methods described in those examples.	
Crystals of a polypeptide having the sequence of SEQ ID NO: 214, prepared and purified as described above and having a His tag, are obtained using the following conditions: sodium citrate 1.4M, Tris-HCl 0.1M, pH 8.5. The crystals were prepared using the following method: 20°C, sitting-drop, 15 mg polypeptide per ml of solution.	
Co-crystals of a polypeptide having the sequence of SEQ ID NO: 214 and adenine, are obtained using the following conditions: sodium citrate 1.4M, Tris-HCl 0.1M, pH 8.5. The concentration of the polypeptide in the solution used to prepare the crystal was 15 mg/mL and the concentration of the ligand was 2 mM. The crystals were prepared using the following method: 20°C, sitting-drop, 15 mg polypeptide per ml of solution. The subject crystallized polypeptide contains the His tag described above.	
Co-crystals of a polypeptide having the sequence of SEQ ID NO: 214 and AMP, are obtained using the following conditions: sodium citrate 1.4M, Tris-HCl 0.1M, pH 8.5. The concentration of the polypeptide in the solution used to prepare the crystal was 15 mg/mL and the concentration of the ligand was 2 mM and 10 mM. The crystals were prepared using the following method: 20°C, sitting-drop, 15 mg polypeptide per ml of solution. The subject crystallized polypeptide contains the His tag described above.	
Co-crystals of a polypeptide having the sequence of SEQ ID NO: 214 and 5-phospho-alpha-d-ribose-1-diphosphate sodium salt, are obtained using the following conditions: tri-sodium Citrate dihydrate 1.4M , HEPES 0.1M pH 7.5 . The concentration of the polypeptide in the solution used to prepare the crystal was 15 mg/mL and the concentration of the ligand was 2 mM and 10 mM. The crystals were prepared using the following method: 20°C, sitting-drop, 15 mg polypeptide per ml of solution. The subject crystallized polypeptide contains the His tag described above.	

**FIGURE 181****TABLE 37 Bioinformatic Analyses of adenine phosphoribosyltransferase from *S. aureus***

TABLE 37 -- adenine phosphoribosyltransferase from <i>S. aureus</i> -- SEQ ID NO: 211-SEQ ID NO: 214	
COG Category	Nucleotide Transport and Metabolism
COG ID Number	COG0503
Is SEQ ID NO: 212 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 212	Adenine Phosphoribosyltransferase, (1qcd)
Source organism for closest PDB protein to SEQ ID NO: 212	<i>Leishmania donovani</i>
e-value for closest PDB Protein to SEQ ID NO: 212	7E-15
% Identity between SEQ ID NO: 212 and the closest protein from PDB	31
% Positives between SEQ ID NO: 212 and the closest protein from PDB	48
Number of Protein Hits in the VGDB to SEQ ID NO: 212	11
Number of Microorganisms having VGDB Hits to SEQ ID NO: 212	11
Microorganisms having VGDB Hits to SEQ ID NO: 212 <sup>1</sup>	[hinf][efae][spne][saur][bsub][ecoli][bbur][hpyl][paer][mgen][nmen]
First predicted epitopic region of SEQ ID NO: 212: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 217 :GFIIGCPVAYS, 1.161, 59->69
Second predicted epitopic region of SEQ ID NO: 212: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 218 :VDIVVGP, 1.158, 49->55
Third predicted epitopic region of SEQ ID NO: 212: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 219 :EAAIKLVEKLG-GIVVGIAFIHELKYL, 1.157, 128->153

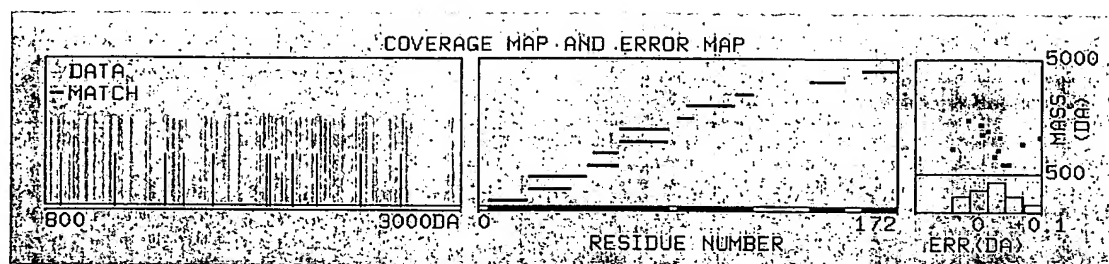
- 5                   <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Eschericia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpax* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

FIGURE 182

Measured peptides : 73

Matched peptides : 12

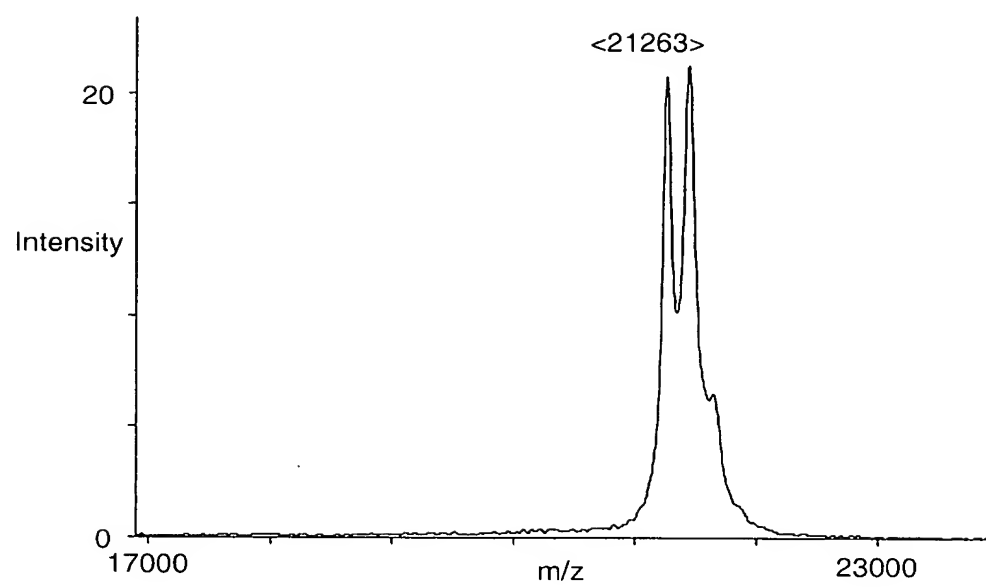
Min. sequence coverage: 78%



Measured Avg/ Computed Error Residues Missed

Mass(M)	Mono	Mass	(Da)	Start	To	Cut	Peptide sequence
881.606	M	881.544	0.062	83	89	1	LPREVIR
883.560	M	883.487	0.073	106	113	0	DAIKPGQR
1168.647	M	1168.608	0.039	48	58	0	DVDIVVGPEAR
1439.786	M	1439.736	0.049	46	58	1	DRDVDIVVGPEAR
1540.898	M	1540.958	-0.061	137	151	0	LGGIVVGIAFIIEK
1689.884	M	1689.780	0.104	159	172	1	IKDYDVMSLSIYDE
1977.015	M	1976.866	0.149	22	39	0	DITTIMDNGEAYGYATDK
1993.045	M	1992.994	0.051	5	21	0	QYVSEVQDWPKPGVSFK
2111.080	M	2111.069	0.011	59	78	0	GFIIGCPVAYSMGIGFAPVR
2239.184	M	2239.164	0.021	59	79	1	GFIIGCPVAYSMGIGFAPVRK
2472.205	M	2472.198	0.007	86	105	1	EVIRYEYDLEYGTNVLTMHK
2680.232	M	2680.256	-0.025	22	45	1	DITTIMDNGEAYGYATDKIVEYAK

FIGURE 183



**FIGURE 184**

SEQ ID NO: 220

GTGTCTTCTTTACTTGTATATGTTACATATATTCACGATAGAGAGGATAA  
5 GAAAATGGCTCAAATTTCTAAATATAAACGTGTAGTTTTGAACTAAGTGGTGA  
AGCGTTAGCTGGAGAAAAAGGATTTGGCATAAATCCAGTAATTATTAAGTG  
TTGCTGAGCAAGTGGCTGAAGTTGCTAAAATGGACTGTGAAATCGCAGTAATC  
GTTGGTGGCGGAAACATTTGGAGAGGTAAAACAGGTAGTGACTTAGGTATGGA  
CCGTGGAAGTCTGATTACATGGGTATGCTTGCAACTGTAATGAATGCCTTAGC  
10 ATTACAAGATAGTTTAGAACAATTGGATTGTGATACACGAGTATTAACATCTAT  
TGAAATGAAGCAAGTGGCTGAACCTTATATTCGTCGTCGTGCAATTAGACACTT  
AGAAAAGAAACGCGTAGTTATTTTTGCTGCAGGTATTGGAAACCCATACTTCTC  
TACAGATACTACAGCGGCATTACGTGCTGCAGAAGTTGAAGCAGATGTTATTTT  
AATGGGCAAAAATAATGTAGATGGTGTATATTCTGCAGATCCTAAAGTAAACA  
15 AAGATGCGGTAAAATATGAACATTTAACGCATATTCAAATGCTTCAAGAAGGT  
TTACAAGTAATGGATTCAACAGCATCCTCATTCTGTATGGATAATAACATTCCG  
TTAACTGTTTTCTCTATTATGGAAGAAGGAAATATTAAACGTGCTGTTATGGGT  
GAAAAGATAGGTACGTTAATTACAAAATAA

**FIGURE 185**

SEQ ID NO: 221

VSSLLVYVTYIHDREDKKMAQISKYKRVVLKLSGEALAGEKGFGINPVIIKS  
5 VAEQVAEVAKMDCEIAVIVGGGNIWRGKTGSDLGMDRGTADYMGMLATVMNAL  
ALQDSLEQLDCDTRVLTSIEMKQVAEPYIRRRAIRHLEKKRVVIFAAGIGNPYFSTD  
TTAALRAAEVEADVILMGKNNVDGVYSADPKVNKDAVKYEHLTHIQMLQEGLQV  
MDSTASSFCMDNNIPLTVFSIMEEGNIKRAVMGEKIGTLITK



**FIGURE 186**

SEQ ID NO: 222

GTGTCTTCTTTACTTGTATATGTTACATATATTCACGATAGAGAGGATAA  
5 GAAAATGGCTCAAATTTCTAAATATAAACGTGTAGTTTTGAAACTAAGTGGTGA  
AGCGTTAGCTGGAGAAAAAGGATTTGGCATAAATCCAGTAATTATTAAGTG  
TTGCTGAGCAAGTGGCTGAAGTTGCTAAAATGGACTGTGAAATCGCAGTAATC  
GTTGGTGGCGGAAACATTTGGAGAGGTAAACCAGGTAGTGACTTAGGTATGGA  
CCGTGGAAGTCTGATTACATGGGTATGCTTGCAACTGTAATGAATGCTTTAGC  
10 ATTACAAGATAGTTTAGAACAATTGGATTGTGATACACGAGTATTAACATCTAT  
TGAAATGAAGCAAGTGGCTGAACCTTATATTCGTCGTCGTGCAATTAGACACTT  
AGAAAAGAAACGCGTAGTTATTTTTGCTGCAGGTATTGGAAACCCATACTTCTC  
TACAGATACTACAGCGGCATTACGTGCTGCAGAAGTTGAAGCAGATGTTATTTT  
AATGGGCAAAAATAATGTAGATGGTGTATATTCTGCAGATCCTAAAGTAAACA  
15 AAGATGCGGTAAAATATGAACATTTAACGCATATTCAAATGCTTCAAGAAGGT  
TTACAAGTAATGGATTCAACAGCATCCTCATTCTGTATGGATAATAACATTCCG  
TTAACTGTTTTCTCTATTATGGAAGAAGGAAATATTAAACGTGCTGTTATGGGT  
GAAAAGATAGGTACGTTAATTACAAAATAA

**FIGURE 187**

SEQ ID NO: 223

VSSLLVYVITYHDREDKKMAQISKYKRVLKLSGEALAGEKGFGINPVIKS  
5 VAEQVAEVAKMDCEIAVIVGGGNIWRGKPGSDLGMDRGTADYMGMLATVMNAL  
ALQDSLEQLDCDTRVLTSIEMKQVAEPYIRRRAIRHLEKKRVVIFAAGIGNPYFSTD  
TTAALRAAEVEADVILMGKNNVDGVYSADPKVNKDAVKYEHLTHIQMLQEGLQV  
MDSTASSFCMDNNIPLTVFSIMEEGNIKRAVMGEKIGTLITK

**FIGURE 188**

SEQ ID NO: 224

Forward PCR Primer

5 GCGGCGGCCCATATGTCTTCTTTACTTGTATATGTTAC

SEQ ID NO: 225

Reverse PCR Primer

10 GCGCGGATCCTTTTGTAATTAACGTACCTATCTTTTC

**FIGURE 189****TABLE 38 Properties of uridylate kinase from *S. aureus***

TABLE 38 -- uridylate kinase from <i>S. aureus</i> -- SEQ ID NO: 220-SEQ ID NO: 223	
Melting temperature (°C) of SEQ ID NO: 224 (forward PCR primer)	66
Restriction enzyme for SEQ ID NO: 224 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 225 (reverse PCR primer)	68
Restriction enzyme for SEQ ID NO: 225 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 220	777
Number of amino acid residues in SEQ ID NO: 221	258
Number of different nucleic acid residues between SEQ ID NO: 220 and SEQ ID NO: 222	2
Number of different amino acid residues between SEQ ID NO: 221 and SEQ ID NO: 223	1
Calculated molecular weight of SEQ ID NO: 221 polypeptide (kDa)	28.3
Calculated pI of SEQ ID NO: 221 polypeptide	6.1
Solubility of SEQ ID NO: 223 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one-third
Solubility of SEQ ID NO: 223 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	No detectable expression
Amount of purified polypeptide having SEQ ID NO: 223, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	6.36
Amount of purified <sup>15</sup> N labeled polypeptide having SEQ ID NO: 223, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	3.00
Amount of purified polypeptide having SEQ ID NO: 223 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	21.20
Amount of purified <sup>15</sup> N labeled polypeptide having SEQ ID NO: 223 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	9.30

**FIGURE 189-B**

TABLE 38 -- uridylate kinase from <i>S. aureus</i> -- SEQ ID NO: 220-SEQ ID NO: 223	
Z-score for the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 223, determined as described in EXAMPLE 9	0.88
Number of matched peptides in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 223, determined as described in EXAMPLE 9	5
Minimum sequence coverage in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 223, determined as described in EXAMPLE 9	25
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. The identity of interacting proteins identified by using at least one of the methods described in those examples are: cell division protein FtsA (gi 13700984),~50, and ~60 unidentified proteins	

**FIGURE 190****TABLE 39 Bioinformatic Analyses of uridylate kinase from *S. aureus***

TABLE 39 -- uridylate kinase from <i>S. aureus</i> -- SEQ ID NO: 220-SEQ ID NO: 223	
COG Category	Nucleotide Transport and Metabolism
COG ID Number	COG0528
Is SEQ ID NO: 221 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 221	none
Source organism for closest PDB protein to SEQ ID NO: 221	N/A
e-value for closest PDB Protein to SEQ ID NO: 221	N/A
% Identity between SEQ ID NO: 221 and the closest protein from PDB	N/A
% Positives between SEQ ID NO: 221 and the closest protein from PDB	N/A
Number of Protein Hits in the VGDB to SEQ ID NO: 221	12
Number of Microorganisms having VGDB Hits to SEQ ID NO: 221	12
Microorganisms having VGDB Hits to SEQ ID NO: 221 <sup>1</sup>	[saur][efae][ecoli][paer][hinf][spne][bsub][rpxx][nmen][ctra][hpyl][mgen]
First predicted epitopic region of SEQ ID NO: 221: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 226 :LLVYVTYI, 1.214, 4->11
Second predicted epitopic region of SEQ ID NO: 221: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 227 :KMDCEIAVIVGG, 1.200, 62->73
Third predicted epitopic region of SEQ ID NO: 221: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 228 :ISKYKRVVLKLSGE, 1.155, 22->35,

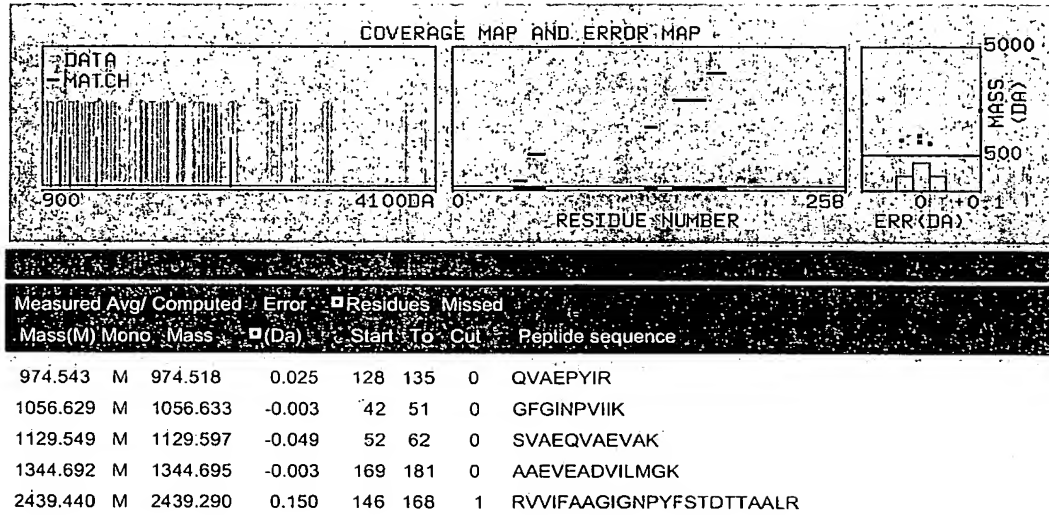
- <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Eschericia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpxx* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

FIGURE 191

Measured peptides: 62

Matched peptides: 5

Min. sequence coverage: 25%



**FIGURE 192**

SEQ ID NO: 229

ATGGCAGACCGAGGCTTACTAATCGTTTTTTCTGGTCCTTCAGGGGTTGG  
5 AAAAGGAACGGTTAGAAGAGAGATTTTTGAGAGTTCTGAAAACCAATTTCAAT  
ATTCTGTATCGATGACGACACGCGCACAAACGTCCTGGAGAAGTGGACGGTGTT  
GACTATTTCTTCCGTACTCGTGAAGAATTTGAAGAGCTGATTCGTCAAGGACAG  
ATGTTGGAATACGCAGAATATGTCGGTAACTACTATGGAACTCCTCTGACCTAT  
GTCAATGAAACCTTGGACAAGGGAATCGATGTTTTCTTGAAATTGAAGTTCAG  
10 GGTGCTCTTCAGGTCAAGAAAAAGGTTCCAGATGCTGTCTTTATCTTCCTGACA  
CCACCAGATTTGGATGAATTGCAAGATCGCTTGGTAGGTCGTGGAACAGATAG  
TGCAGAAGTGATTGCCCAACGAATCGAAAAGGCCAAGGAAGAAATTGCCCTCA  
TGCGTGAGTATGATTATGCGATTGTCAACGATCAGGTACCCCTAGCTGCTGAAC  
GTGTCAAATGTGTGATTGAAGCAGAACACTTCTGTGTGGATCGTGTCATTGGTC  
15 ACTATCAGGAGATGTTACCAAAATCTCCAACCTACCCGATAA



**FIGURE 193**

SEQ ID NO: 230

MADRGLLIVFSGPSGVGKGTVRREIFESSENQFQYSVSMTTTRAQRPGEVDG  
5 VDYFFRTREEFEELIRQGQMLEYAEYVGNYGTPLTYVNETLDKGIDVFLEIEVQG  
ALQVKKKVPDAVFIFLTPPDLDELQDRLVGRGTDSAEVIAQRIEKAKEEIALMREY  
DYAIVNDQVPLAAERVKCVIEAEHFCVDRVIGHYQEMLPKSPTTR

**FIGURE 194**

SEQ ID NO: 231

ATGGCAGACCGAGGCTTACTAATCGTTTTTTCTGGTCCTTCAGGGGTTGG  
5 AAAAGGAACGGTTAGAAGAGAGATTTTTGAGAGTTCTGAAAACCAATTTCAAT  
ACTCTGTATCGATGACGACACGCGCACAAACGTCCTGGAGAAGTGGACGGTGTT  
GACTATTTCTTCCGTACTCGTGAAGAATTTGAAGAGCTGATTCGTCAAGGACAG  
ATGTTGGAATACGCAGAATATGTCGGCAACTACTATGGAACCTCCTCTGACCTAT  
GTCAATGAAACCTTGGACAAGGGAATCGATGTTTTCTTGAAATTGAAGTTCAG  
10 GGTGCTCTTCAGGTCAAGAAAAAGGTTCCAGATGCTGTCTTTATCTTCCTGACA  
CCACCAGATTTGGATGAATTGCAAGATCGCTTGGTAGGTCGTGGAACAGATAG  
TGCAGAAGTGATTGCCCAACGAATCGAAAAGGCCAAGGAAGAAATTGCCCTCA  
TGCGTGAGTATGATTATGCGATTGTCAACGATCAGGTACCCCTAGCTGCTGAAC  
GTGTCAAATGTGTGATTGAAGCAGAACACTTCTGTGTGGATCGTGTCATTGGTC  
15 ACTATCAGGAGATGTTACCAAAATCTCCAACCTACCCGATAA

**FIGURE 195**

SEQ ID NO: 232

MADRGLLIVFSGPSGVGKGTVRREIFESSENQFQYSVSMTTRAQRPGEVDG  
5 VDYFFRTREEFEELIRQGQMLEYAEYVGNYYGTPLTYVNETLDKGIDVFLEIEVQG  
ALQVKKKVPDAVFIFLTPPDLDELQDRLVGRGTDSAEVIAQRIEKAKEEIALMREY  
DYAIVNDQVPLAAERVKCVIEAEHFCVDRVIGHYQEMLPKSPTTR

**FIGURE 196**

SEQ ID NO: 233

Forward PCR Primer

5 GCGGCGGCCCATATGGCAGACCGAGGCTTAC

SEQ ID NO: 234

10

Reverse PCR Primer

GCGCGGATCCTCGGGTAGTTGGAGATTTTG

**FIGURE 197****TABLE 40 Properties of guanylate kinase from *S. pneumoniae***

TABLE 40 -- guanylate kinase from <i>S. pneumoniae</i> -- SEQ ID NO: 229-SEQ ID NO: 232	
Melting temperature (°C) of SEQ ID NO: 233 (forward PCR primer)	60
Restriction enzyme for SEQ ID NO: 233 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 234 (reverse PCR primer)	58
Restriction enzyme for SEQ ID NO: 234 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 229	627
Number of amino acid residues in SEQ ID NO: 230	208
Number of different nucleic acid residues between SEQ ID NO: 229 and SEQ ID NO: 231	2
Number of different amino acid residues between SEQ ID NO: 230 and SEQ ID NO: 232	0
Calculated molecular weight of SEQ ID NO: 230 polypeptide (kDa)	23.7
Calculated pI of SEQ ID NO: 230 polypeptide	4.5
Solubility of SEQ ID NO: 232 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching 100%
Solubility of SEQ ID NO: 232 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	Approaching 100%
Amount of purified polypeptide having SEQ ID NO: 232, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	47.2
Amount of purified selmet labeled polypeptide having SEQ ID NO: 232, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	25.1
Amount of purified <sup>15</sup> N labeled polypeptide having SEQ ID NO: 232, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	19.9
Amount of purified polypeptide having SEQ ID NO: 232 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	7.5
Amount of purified selmet labeled polypeptide having SEQ ID NO: 232 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	71.8
Amount of purified <sup>15</sup> N labeled polypeptide having SEQ ID NO: 232 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	44.2

**FIGURE 197-B**

TABLE 40 -- guanylate kinase from <i>S. pneumoniae</i> -- SEQ ID NO: 229-SEQ ID NO: 232	
Z-score for the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 232, determined as described in EXAMPLE 9	2.1E-8
Number of matched peptides in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 232, determined as described in EXAMPLE 9	14
Minimum sequence coverage in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 232, determined as described in EXAMPLE 9	84
Calculated molecular weight of SEQ ID NO: 230 polypeptide (Da), determined as described in EXAMPLE 10	25717
Experimental molecular weight of SEQ ID NO: 232 polypeptide (Da), determined as described in EXAMPLE 10	25952
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. The identity of an interacting protein identified by using at least one of the methods described in those examples is: ~45 unidentified protein.	
Crystals of a polypeptide having the sequence of SEQ ID NO: 232, prepared and purified as described above and having a His tag, are obtained using the following conditions: PEG 400 35%, sodium cacodylate 0.1M, pH 6.5, 0.2M calcium acetate. In addition, crystals of the same polypeptide may be prepared under the following conditions: PEG 1500 30%, sodium cacodylate 0.1M, pH 6.5, 0.2M sodium chloride. Further, crystals of the same polypeptide may be prepared under the following conditions: PEG 4000 30%, sodium cacodylate 0.1M, pH 6.5, 0.2M sodium acetate. The crystals were prepared using the following method: 20°C, sitting-drop, 15 mg polypeptide per ml of solution.	
Crystals of a selenomethionine-substituted polypeptide having the sequence of SEQ ID NO: 232, prepared and purified as described above and having a His tag, are obtained using the following conditions: ammonium sulfate 2M , Tris 0.1M pH 8.5. In addition, crystals of the same polypeptide may be prepared under the following conditions: ammonium sulfate 2M , HEPES 0.1M pH 7.5, PEG400 2%. Further, crystals of the same polypeptide may be prepared under the following conditions: sodium citrate 1.4M, sodium acetate 0.1M, pH 4.5. Further, crystals of the same polypeptide may be prepared under the following conditions: sodium formate 2M , sodium acetate 0.1M pH 4.6 . The crystals were prepared using the following method: 20°C, sitting-drop, 15 mg polypeptide per ml of solution.	

FIGURE 197-C

TABLE 40 -- guanylate kinase from *S. pneumoniae* -- SEQ ID NO: 229-SEQ ID NO: 232

Co-crystals of a polypeptide having the sequence of SEQ ID NO: 232 and ATP, are obtained using the following conditions: ammonium sulfate 2M , Tris 0.1M pH 8.5. Further, crystals may be obtained under the following conditions: ammonium sulfate 2M, HEPES 0.1M pH 7.5, PEG 400 2%. The concentration of the polypeptide in the solution used to prepare the crystal was 15 mg/mL and the concentration of the ligand was 2. The crystals were prepared using the following method: 20°C, sitting-drop, 15 mg polypeptide per ml of solution. The subject crystallized polypeptide contains the His tag described above.

Co-crystals of a polypeptide having the sequence of SEQ ID NO: 232 and GMP, are obtained using the following conditions: ammonium sulfate 2M, Tris 0.1M pH 8.5. The concentration of the polypeptide in the solution used to prepare the crystal was 15 mg/mL and the concentration of the ligand was 2 mM. The crystals were prepared using the following method: 20°C, sitting-drop, 15 mg polypeptide per ml of solution. The subject crystallized polypeptide contains the His tag described above.

Co-crystals of a polypeptide having the sequence of SEQ ID NO: 232 and GDP, are obtained using the following conditions: PEG 4000 30% , tri-sodium citrate dihydrate 0.1M pH 5.6, ammonium acetate 0.2M. The concentration of the polypeptide in the solution used to prepare the crystal was 15 mg/ml and the concentration of the ligand was 2 and 10 mM. The crystals were prepared using the following method: 20°C, sitting-drop, 15 mg polypeptide per ml of solution. The subject crystallized polypeptide contains the His tag described above.

**FIGURE 198****TABLE 41 Bioinformatic Analyses of guanylate kinase from *S. pneumoniae***

TABLE 41 -- guanylate kinase from <i>S. pneumoniae</i> -- SEQ ID NO: 229-SEQ ID NO: 232	
COG Category	Nucleotide Transport and Metabolism
COG ID Number	COG0194
Is SEQ ID NO: 230 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 230	Guanylate Kinase (1gky)
Source organism for closest PDB protein to SEQ ID NO: 230	<i>Escherichia coli</i>
e-value for closest PDB Protein to SEQ ID NO: 230	1E-32
% Identity between SEQ ID NO: 230 and the closest protein from PDB	39
% Positives between SEQ ID NO: 230 and the closest protein from PDB	61
Number of Protein Hits in the VGDB to SEQ ID NO: 230	13
Number of Microorganisms having VGDB Hits to SEQ ID NO: 230	12
Microorganisms having VGDB Hits to SEQ ID NO: 230 <sup>1</sup>	[spne][bsub][ecoli][efae][saur][hinf][paer][nmen][rpxx][mgen][hpyl][ctra]
First predicted epitopic region of SEQ ID NO: 230: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 235 :DYAIVNDQVPLAAERV-KCVIEAEHFCVDRVIGH, 1.168,164->196
Second predicted epitopic region of SEQ ID NO: 230: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 236 :RGLLIVFSGPSGV, 1.145,4->16
Third predicted epitopic region of SEQ ID NO: 230: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 237 :GIDVFLEIEVQGA-LQVKKKVPDAVFIFLTPP, 1.143,96->126

<sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Escherichia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpxx* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

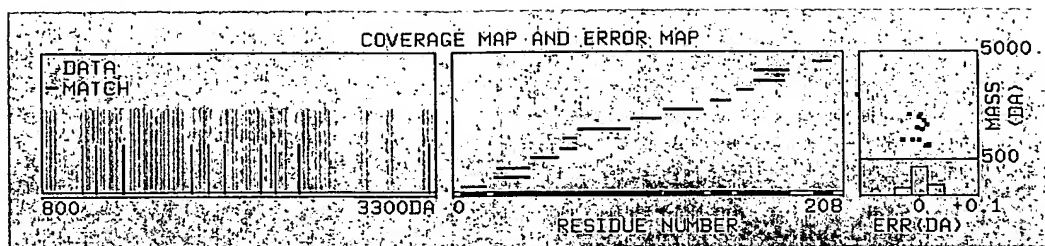


FIGURE 199

Measured peptides: 67

Matched peptides: 14

Min. sequence coverage: 84%

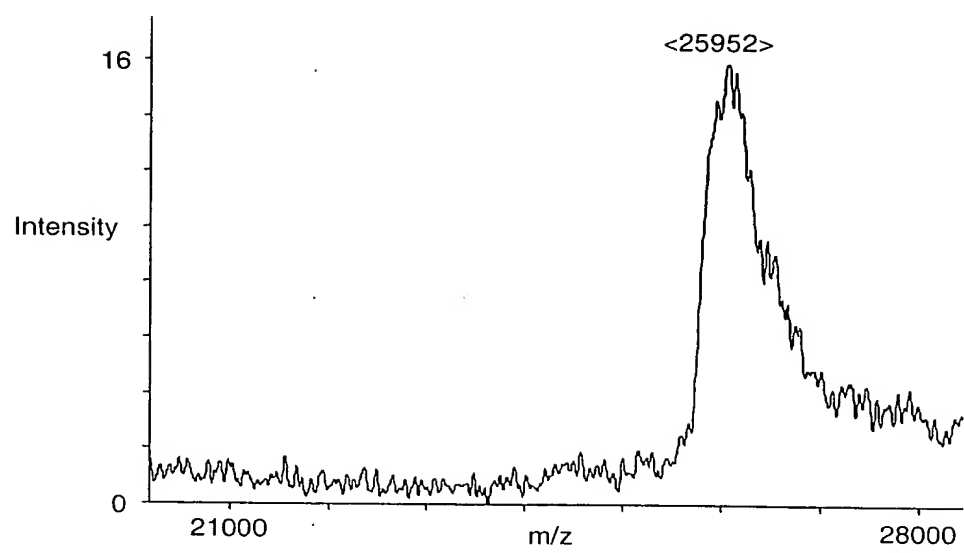


Measured Avg/ Computed Error Residues Missed

Mass(M) Mono Mass (Da) Start To Cut Peptide sequence

1059.588	M	1059.574	0.014	153	161	1	AKEEIALMR
1063.539	M	1063.518	0.021	60	67	0	EEFEELIR
1145.589	M	1145.567	0.022	139	149	0	GTDSAEVIAQR
1313.681	M	1313.679	0.001	193	203	0	VIGHYQEMLPK
1320.655	M	1320.667	-0.011	58	67	1	TREEFEELIR
1329.734	M	1329.765	-0.031	5	18	0	GLLIVFSGPSGVGK
1754.844	M	1754.837	0.007	43	57	0	AQRPGEVGDGVDFFR
1857.022	M	1857.024	-0.002	96	112	0	GIDVFLEIEVQGALQVK
1964.966	M	1964.947	0.019	162	178	0	EYDYAIVNDQVPLAAER
2192.121	M	2192.110	0.011	162	180	1	EYDYAIVNDQVPLAAERVK
2282.017	M	2282.015	0.002	24	42	0	EIFESSENQFQYSVSMTTR
2427.272	M	2427.268	0.004	114	134	1	KVPDAVFIFLTPPDLDELQDR
2438.098	M	2438.116	-0.018	23	42	1	REIFESSENQFQYSVSMTTR
3258.385	M	3258.505	-0.121	68	95	0	QGQMLEYAEYVGNYGTPLTYVNETLDK

FIGURE 200



**FIGURE 201**

SEQ ID NO: 238

ATGAATTTAAAAGATTACATTGCAACAATTGAAAATTATCCAAAGGAAG  
5 GCATTACCTTCCGTGATATTAGTCCTTTGATGGCTGATGGAAATGCTTATAGCT  
ACGCTGTTCGTGAAATCGTTCAGTATGCTACTGACAAGAAAGTCGACATGATCG  
TGGGACCTGAAGCTCGTGGATTTATCGTGGGTTGTCCAGTTGCCTTTGAGTTGG  
GAATTGGTTTTTGC GCCTGTTTCGTAAGCCAGGTAAATTGCCACGCGAAGTTATTT  
CTGCTGACTATGAAAAAGAGTACGGTGTCGATACCTTGACTATGCACGCGGAT  
10 GCCATTAAGCCAGGTCAACGTGTTCTTATTGTAGATGACCTTTTGGCGACAGGT  
GGAAGTGTTAAGGCAACTATCGAGATGATTGAAAACTTGGTGGTGTATGGC  
AGGTTGTGCCTTCCTTGTTGAATTGGATGAATTGAACGGCCGTGAAAAAATTGG  
TGACTACGACTACAAAGTTCTTATGCATTATTAA

**FIGURE 202**

SEQ ID NO: 239

MNLKDYIATIENYPKEGITFRDISPLMADGNAYS AVREIVQYATDKKVDMI  
5 VGPEARGFIVGCPVAFELGIGFAPVRKPGKLPREVISADYEKEYGVDTLTMHADAI  
KPGQRVLIVDDLLATGGTVKATIEMIEKLGGVMAGCAFLVELDELNGREKIGDYD  
YKVLMHY

**FIGURE 203**

SEQ ID NO: 240

ATGAATTTAAAAGATTACATTGCAACAATTGAAAATTATCCAAAGGAAG  
5 GCATTACCTTCCGTGATATTAGTCCTTTGATGGCTGATGGAAATGCTTATAGCT  
ACGCTGTTCGTGAAATCGTTCAGTATGCTACTGACAAGAAAGTCGACATGATCG  
TGGGACCTGAAGCTCGTGGATTTATCGTGGGTTGTCCAGTTGCCTTTGAGTTGG  
GAATTGGTTTTTGC GCCTGTTTCGTAAGCCAGGTAAATTGCCACGCGAAGTTATTT  
CTGCTGACTATGAAAAAGAGTACGGTGTCGATACTTTGACTATGCACGCGGATG  
10 CCATTAAGCCAGGTCAACGTGTTCTTATTGTAGATGACCTTTTGGCGACAGGTG  
GAACTGTTAAGGCAACTATCGAGATGATTGAAAACTTGGTGGTGTTATGGCA  
GGTTGTGCCTTCCTTGTTGAATTGGATGAATTGAACGGCCGTGAAAAAATTGGT  
GACTACGACTACAAAGTTCTTATGCATTATTAA

**FIGURE 204**

SEQ ID NO: 241

MNLKDYIATIENYPKEGITFRDISPLMADGNAYS AVREIVQYATDKKVDMI  
5 VGPEARGFIVGCPVAFELGIGFAPVRKPGKLPREVISADYEKEYGVDTLTMHADAI  
KPGQRVLIVDDLLATGGTVKATIEMIEKLGGVMAGCAFLVELDELNGREKIGDYD  
YKVLMHY

**FIGURE 205**

SEQ ID NO: 242

Forward PCR Primer

5 GCGGCGGCCCATATGAATTTAAAAGATTACATTGCAAC

SEQ ID NO: 243

10

Reverse PCR Primer

GCGCGGATCCATAATGCATAAGAACTTTGTAGTC

**FIGURE 206****TABLE 42 Properties of adenine phosphoribosyltransferase from *S. pneumoniae***

TABLE 42 -- adenine phosphoribosyltransferase from <i>S. pneumoniae</i> -- SEQ ID NO: 238-SEQ ID NO: 241	
Melting temperature (°C) of SEQ ID NO: 242 (forward PCR primer)	64
Restriction enzyme for SEQ ID NO: 242 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 243 (reverse PCR primer)	62
Restriction enzyme for SEQ ID NO: 243 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 238	513
Number of amino acid residues in SEQ ID NO: 239	170
Number of different nucleic acid residues between SEQ ID NO: 238 and SEQ ID NO: 240	1
Number of different amino acid residues between SEQ ID NO: 239 and SEQ ID NO: 241	0
Calculated molecular weight of SEQ ID NO: 239 polypeptide (kDa)	18.7
Calculated pI of SEQ ID NO: 239 polypeptide	4.7
Solubility of SEQ ID NO: 241 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approximately two-thirds
Solubility of SEQ ID NO: 241 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	Approaching one-third
Amount of purified polypeptide having SEQ ID NO: 241, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	36.3
Amount of purified selmet labeled polypeptide having SEQ ID NO: 241, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	11.1
Amount of purified <sup>15</sup> N labeled polypeptide having SEQ ID NO: 241, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	35.3
Amount of purified polypeptide having SEQ ID NO: 241 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	67.2
Amount of purified selmet labeled polypeptide having SEQ ID NO: 241 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	11.1
Amount of purified <sup>15</sup> N labeled polypeptide having SEQ ID NO: 241 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	25.5



**FIGURE 206-B**

TABLE 42 -- adenine phosphoribosyltransferase from <i>S. pneumoniae</i> -- SEQ ID NO: 238-SEQ ID NO: 241	
Z-score for the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 241, determined as described in EXAMPLE 9	1.5E-6
Number of matched peptides in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 241, determined as described in EXAMPLE 9	13
Minimum sequence coverage in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 241, determined as described in EXAMPLE 9	91
Calculated molecular weight of SEQ ID NO: 239 polypeptide (Da), determined as described in EXAMPLE 10	20760
Experimental molecular weight of SEQ ID NO: 241 polypeptide (Da), determined as described in EXAMPLE 10	20919
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. No interacting proteins were observed by using at least one of the methods described in those examples.	
Crystals of a polypeptide having the sequence of SEQ ID NO: 241, prepared and purified as described above and having a His tag, are obtained using the following conditions: PEG 400 35%, sodium cacodylate 0.1M, pH 6.5, 0.2M calcium acetate. In addition, crystals of the same polypeptide may be prepared under the following conditions: PEG 1500 30%. Further, crystals of the same polypeptide may be prepared under the following conditions: PEG 1500 30%, sodium cacodylate 0.1M, pH 6.5, 0.2M sodium chloride. Further, crystals of the same polypeptide may be prepared under the following conditions: PEG 4000 30%, sodium citrate 0.1M, pH 5.5, 0.2M ammonium acetate. Still further crystals of the same polypeptide may be prepared under the following conditions: PEG 4000 30%, sodium cacodylate 0.1M, pH 6.5, 0.2M sodium acetate. The crystals were prepared using the following method: 20°C, sitting-drop, 15 mg polypeptide per ml of solution.	

FIGURE 206-C

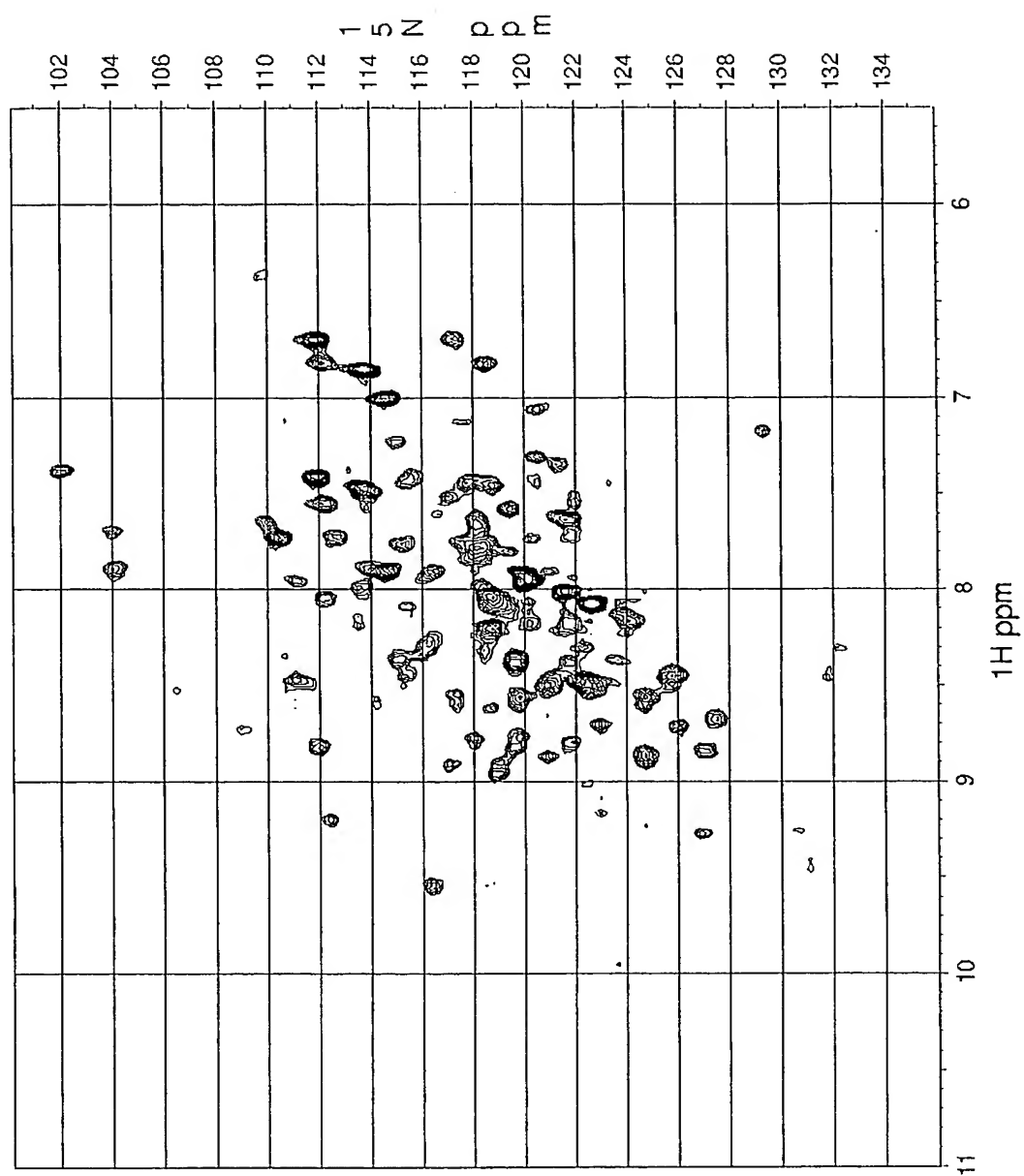
TABLE 42 -- adenine phosphoribosyltransferase from <i>S. pneumoniae</i> -- SEQ ID NO: 238-SEQ ID NO: 241
Crystals of a selenomethionine-substituted polypeptide having the sequence of SEQ ID NO: 241, prepared and purified as described above and having a His tag, are obtained using the following conditions: PEG 400 35%, sodium cacodylate 0.1M, pH 6.5, 0.2M calcium acetate. In addition, crystals of the same polypeptide may be prepared under the following conditions: PEG 4000 30% , tri-sodium citrate dihydrate 0.1M pH 5.6, ammonium acetate 0.2M. Further, crystals of the same polypeptide may be prepared under the following conditions: tri-sodium citrate dihydrate 1.4M, HEPES 0.1M pH 7.5. Further, crystals of the same polypeptide may be prepared under the following conditions: sodium citrate 1.4M, Tris-HCl 0.1M, pH 8.5. The crystals were prepared using the following method: 20°C, sitting-drop, 11.9 mg polypeptide per ml of solution.
Co-crystals of a polypeptide having the sequence of SEQ ID NO: 241 and 5-phospho-alpha-d-ribose-1-diphosphate sodium salt, are obtained using the following conditions: PEG 400 35%, sodium cacodylate 0.1M, pH 6.5, 0.2M calcium acetate. Crystals of the same complex may be prepared under the following conditions: PEG 1500 30%, sodium cacodylate 0.1M, pH 6.5, 0.2M sodium chloride. Further, crystals of the same complex may be prepared under the following conditions: PEG 4000 30% , tri-sodium citrate dihydrate 0.1M pH 5.6, ammonium acetate 0.2M. The concentration of the polypeptide in the solution used to prepare the crystal was 15 mg/mL and the concentration of the ligand was 2 and 10 mM. The crystals were prepared using the following method: 20°C, sitting-drop, 15 mg polypeptide per ml of solution. The subject crystallized polypeptide contains the His tag described above.

**FIGURE 207****TABLE 43 Bioinformatic Analyses of adenine phosphoribosyltransferase from *S. pneumoniae***

TABLE 43 -- adenine phosphoribosyltransferase from <i>S. pneumoniae</i> -- SEQ ID NO: 238-SEQ ID NO: 241	
COG Category	Nucleotide Transport and Metabolism
COG ID Number	COG0503
Is SEQ ID NO: 239 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 239	Adenine Phosphoribosyltransferase (1qcd)
Source organism for closest PDB protein to SEQ ID NO: 239	<i>Leishmania donovani</i>
e-value for closest PDB Protein to SEQ ID NO: 239	4E-12
% Identity between SEQ ID NO: 239 and the closest protein from PDB	35
% Positives between SEQ ID NO: 239 and the closest protein from PDB	57
Number of Protein Hits in the VGDB to SEQ ID NO: 239	11
Number of Microorganisms having VGDB Hits to SEQ ID NO: 239	11
Microorganisms having VGDB Hits to SEQ ID NO: 239 <sup>1</sup>	[spne][efae][hinf][saur][bsub][ecoli][bbur][hpyl][paer][mgen][nmen]
First predicted epitopic region of SEQ ID NO: 239: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 244 :GFIVGCPVAFELGIGF-APVRKP, 1.194,59->80
Second predicted epitopic region of SEQ ID NO: 239: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 245 :GVMAGCAFLVELD, 1.186,139->151
Third predicted epitopic region of SEQ ID NO: 239: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 246 :GQRVLIVDDLLATG, 1.164,111->124

- 5                   <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Eschericia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpax* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

FIGURE 208

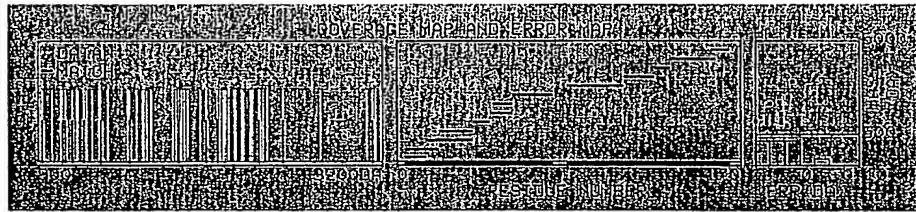


## FIGURE 209

Measured peptides: 55

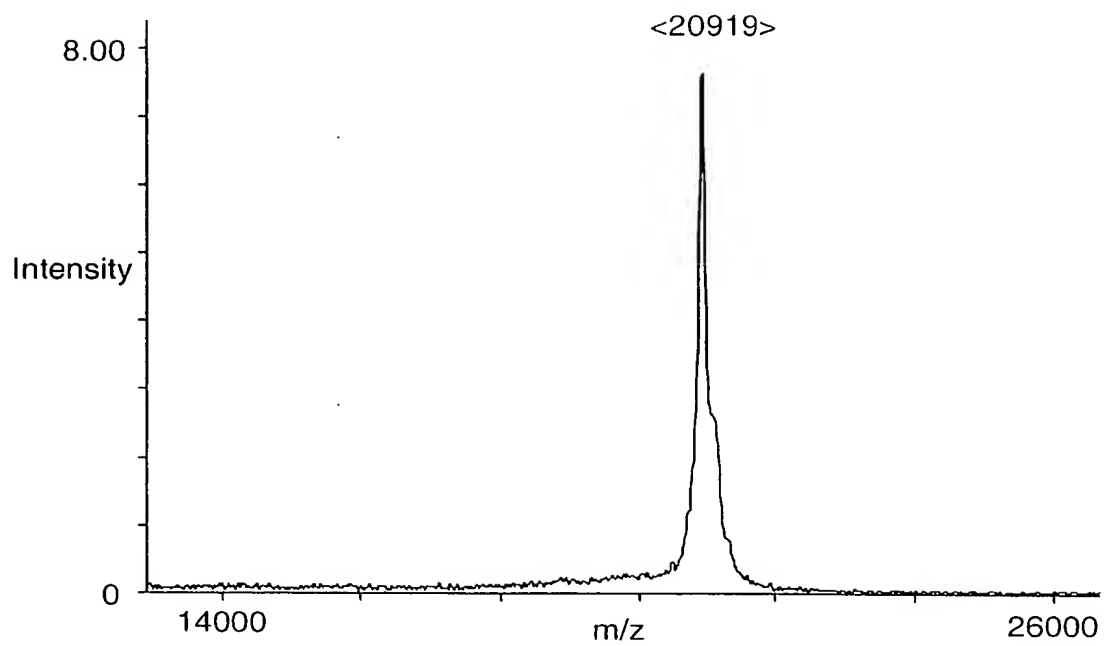
Matched peptides: 13

Min. sequence coverage: 91%



Measured	Avg/	Computed	Error	Residues			Missed
Mass(M)	Mono	Mass	(Da)	Start	To	Cut	Peptide sequence
933.448	M	933.483	-0.035	129	136	0	ATIEMIEK
1065.459	M	1065.534	-0.074	39	47	0	EIVQYATDK
1085.506	M	1085.553	-0.047	49	58	0	VDMIVGPEAR
1129.432	M	1129.529	-0.097	157	165	1	EKIGDYDYK
1213.602	M	1213.648	-0.046	48	58	1	KVDMIVGPEAR
1325.575	M	1325.650	-0.074	5	15	0	DYIATIENYPK
1512.828	M	1512.875	-0.048	114	128	0	VLIVDLLATGGTVK
1841.824	M	1841.861	-0.038	22	38	0	DISPLMADGNAYSAYR
2028.945	M	2029.015	-0.069	5	21	1	DYIATIENYPKEGITFR
2105.062	M	2105.112	-0.050	59	78	0	GFIVGCPVAFELGIGFAPVR
2377.142	M	2377.176	-0.033	137	158	1	LGGVMAGCAFLVELDELNGREK
2545.240	M	2545.226	0.014	16	38	1	EGITFRDISPLMADGNAYSAYR
3135.490	M	3135.517	-0.027	86	113	1	EVISADYEKEYGVDLTLMHADAIKPGQR

FIGURE 210



**FIGURE 211**

SEQ ID NO: 247

GTGAAAATGGCGAATCCCAAGTATAAACGTATTTTAATCAAGTTATCAG  
5 GTGAAGCCCTTGCCGGTGAACGTGGCGTAGGGATTGATATCCAAACAGTTCAA  
ACAATCGCAAAAGAGATTCAAGAAGTTCATAGCTTAGGTATCGAAATTGCCCTT  
GTTATCGGTGGAGGAAATCTCTGGCGTGGAGAACCTGCAGCAGAAGCAGGTAT  
GGACCGTGTTTCAGGCAGATTACACAGGAATGCTTGGGACTGTTATGAATGCTCT  
TGTGATGGCAGATTCATTGCAACAAGTTGGGGTTGATACGCGTGTACAAACAG  
10 CTATTGCCATGCAACAAGTGGCAGAGCCTTATGTCCGTGGACGTGCCCTTCGTC  
ACCTTGAAAAAGGCCGTATCGTTATCTTTGGTGCTGGAATTGGTTCACCTTACTT  
CTCGACAGATAACAACAGCGGCCCTTCGTGCAGCTGAAATCGAAGCAGATGCCA  
TCCTCATGGCTAAAAATGGTGTCGATGGTGTTTACAATGCCGATCCTAAGAAAG  
ATAAGACAGCTGTTAAGTTTGAAGAATTGACCCACCGTGACGTTATCAATAAA  
15 GGTCTTCGTATCATGGACTCAACAGCTTCAACCCTCTCAATGGACAACGACATT  
GACTTGGTTGTATTCAACATGAACCAACCAGGCAACATCAAACGTGTCGTATTT  
GGTGAAAATATCGGAACAACAGTTTCAAATAATATCGAAGAAAAGGAATAA

**FIGURE 212**

SEQ ID NO: 248

VKMANPKYKRILIKLSGEALAGERGVGIDIQTVQTIAKEIQEVHSLGIELALVI  
5 GGGNLWRGEPAAEAGMDRVQADYTGMLGTVMNALVMADSLQQVGVDTTRVQTA  
IAMQQVAEPYVRGRALRHLEKGRIVIFGAGIGSPYFSTDTTAALRAAEIEADAILMA  
KNGVDGVYNADPKKDKTAVKFEELTHRDVINKGLRIMDSTASTLSMDNDIDL VVF  
NMNQPGNIKR VVFGENIGTTVSNNIEEKE



**FIGURE 213**

SEQ ID NO: 249

GTGAAAATGGCGAATCCCAAGTATAAACGTATTTTAATCAAGTTATCAG  
5 GTGAAGCCCTTGCCGGTGAACGTGGCGTAGGGATTGATATCCAAACAGTTCAA  
ACAATCGCAAAAGAGATTCAAGAAGTTCATAGCTTAGGTATCGAAATTGCCCTT  
GTTATTGGTGGAGGAAATCTCTGGCGTGGAGACCCTGCAGCAGAAGCAGGTAT  
GGACCGTGTTCAAGGCAGATTACACTGGAATGCTTGGGACTGTTATGAATGCTCT  
TGTGATGGCAGATTCATTGCAACAAGTTGGGGTTGATACGCGTGTACAAACAG  
10 CTATTGCTATGCAACAAGTGGCAGAGCCTTATGTCCGTGGACGTGCCCTTCGTC  
ACCTTGAAAAAGGCCGTATCGTTATCTTTGGTGCTGGAATTGGTTCACCATACT  
TCTCGACAGATACAACAGCGGCCCTTCGTGCAGCTGAAATCGAAGCAGATGCC  
ATCCTCATGGCTAAAAATGGCGTCGATGGTGTGTACAATGCCGATCCTAAGAA  
GGACAAGACAGCCGTAAAGTTTGAAGAATTGACCCACCGTGATGTTATCAACA  
15 AAGGTCTTCGTATCATGGACTCAACAGCCTCAACCCTCTCAATGGACAACGACA  
TTGACTTGGTTGTCTTCAACATGAACCAATCAGGCAACATCAAACGTGTCGTAT  
TTGGTGAAAATATCGGAACAACAGTTTCAAATAATATCGAAGAAAAGGAATAA

**FIGURE 214**

SEQ ID NO: 250

VKMANPKYKRILIKLSGEALAGERGVGIDIQTVQTIAKEIQEVHSLGIEIALVI  
5 GGGNLWRGDPAAEAGMDRVQADYTGMLGTVMNALVMADSLQQVGVDTRVQT  
AIAMQQVAEPYVRGRALRHLEKGRIVIFGAGIGSPYFSTDTTAALRAAEIEADAILM  
AKNGVDGVYNADPKKDKTAVKFEELTHRDVINKGLRIMDSTASTLSMDNDIDLVV  
FNMNQSGNIKRVVFGENIGTTVSNNIEEKE

**FIGURE 215**

SEQ ID NO: 251

Forward PCR Primer  
5 GCGGCGGCCCATATGAAAATGGCGAATCCCAAG

SEQ ID NO: 252

10 Reverse PCR Primer  
GCGCGGATCCTTCCTTTCTTCGATATTATTG

FIGURE 216

TABLE 44 Properties of uridylate kinase from *S. pneumoniae*

TABLE 44 -- uridylate kinase from <i>S. pneumoniae</i> -- SEQ ID NO: 247-SEQ ID NO: 250	
Melting temperature (°C) of SEQ ID NO: 251 (forward PCR primer)	60
Restriction enzyme for SEQ ID NO: 251 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 252 (reverse PCR primer)	58
Restriction enzyme for SEQ ID NO: 252 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 247	744
Number of amino acid residues in SEQ ID NO: 248	247
Number of different nucleic acid residues between SEQ ID NO: 247 and SEQ ID NO: 249	15
Number of different amino acid residues between SEQ ID NO: 248 and SEQ ID NO: 250	2
Calculated molecular weight of SEQ ID NO: 248 polypeptide (kDa)	26.7
Calculated pI of SEQ ID NO: 248 polypeptide	5.3
Solubility of SEQ ID NO: 250 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approximately two-thirds
Solubility of SEQ ID NO: 250 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	Approaching one-third
Amount of purified polypeptide having SEQ ID NO: 250, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	26.0
Amount of purified selmet labeled polypeptide having SEQ ID NO: 250, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	25.8
Amount of purified <sup>15</sup> N labeled polypeptide having SEQ ID NO: 250, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	59.5
Amount of purified polypeptide having SEQ ID NO: 250 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	90.0
Amount of purified selmet labeled polypeptide having SEQ ID NO: 250 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	47.7
Amount of purified <sup>15</sup> N labeled polypeptide having SEQ ID NO: 250 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	41

**FIGURE 216-B**

TABLE 44 -- uridylate kinase from <i>S. pneumoniae</i> -- SEQ ID NO: 247-SEQ ID NO: 250	
Calculated molecular weight of SEQ ID NO: 248 polypeptide (Da), determined as described in EXAMPLE 10	28701
Experimental molecular weight of SEQ ID NO: 250 polypeptide (Da), determined as described in EXAMPLE 10	28997
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. The identity of an interacting protein identified by using at least one of the methods described in those examples is: DNA-binding protein HU (gi 14972589).	
Crystals of a polypeptide having the sequence of SEQ ID NO: 250, prepared and purified as described above and having a His tag, are obtained using the following conditions: tri-sodium citrate dihydrate 1.4M, HEPES 0.1M pH 7.5. The crystals were prepared using the following method: 20°C and 4°C, sitting-drop, 10 mg polypeptide per ml of solution.	
Crystals of a selenomethionine-substituted polypeptide having the sequence of SEQ ID NO: 250, prepared and purified as described above and having a His tag, are obtained using the following conditions: ammonium sulfate 2M, HEPES 0.1M pH 7.5, PEG 400 2%. In addition, crystals of the same polypeptide may be prepared under the following conditions: tri-sodium citrate dihydrate 1.4M, HEPES 0.1M pH 7.5. Further, crystals of the same polypeptide may be prepared under the following conditions: sodium citrate 1.4M, Tris-HCl 0.1M, pH 8.5. The crystals were prepared using the following method: 20°C, sitting-drop, 15 mg polypeptide per ml of solution.	
Co-crystals of a polypeptide having the sequence of SEQ ID NO: 250 and L-methionine, are obtained using the following conditions: tri-sodium citrate dihydrate 1.4M, HEPES 0.1M pH 7.5. The concentration of the polypeptide in the solution used to prepare the crystal was 15 mg/mL and the concentration of the ligand was 2 and 10 mM. The crystals were prepared using the following method: 20°C, sitting-drop, 15 mg polypeptide per ml of solution. The subject crystallized polypeptide contains the His tag described above.	
Co-crystals of a polypeptide having the sequence of SEQ ID NO: 250 and UDP, are obtained using the following conditions: ammonium dihydrogen phosphate 1.0M. The concentration of the polypeptide in the solution used to prepare the crystal was 15 mg/mL and the concentration of the ligand was 2 mM. The crystals were prepared using the following method: 20°C, sitting-drop, 15 mg polypeptide per ml of solution. The subject crystallized polypeptide contains the His tag described above.	
Co-crystals of a polypeptide having the sequence of SEQ ID NO: 250 and UMP, are obtained using the following conditions: tri-sodium citrate dihydrate 1.4M, HEPES 0.1M pH 7.5. The concentration of the polypeptide in the solution used to prepare the crystal was 15 mg/mL and the concentration of the ligand was 2 mM. The crystals were prepared using the following method: 20°C, sitting-drop, 15 mg polypeptide per ml of solution. The subject crystallized polypeptide contains the His tag described above.	

**FIGURE 216-C**TABLE 44 continued: Truncation Polypeptides of uridylylate kinase from *S. pneumoniae*

Start of truncated polypeptide of SEQ ID NO: 250	M3	M3	M3
End of truncated polypeptide of SEQ ID NO: 250	V239	N241	I243
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approximately two-thirds	Approaching 100%	Approximately two-thirds
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	No discernable expression		No discernable expression
Amount of purified truncated polypeptide, prepared and purified as described in the Exemplification (mg/L of culture).	27.9	38.8	68.3
Amount of purified, truncated polypeptide soluble in buffer, as described in the Exemplification (mg/ml of buffer)	32.4	38.8	48.8
Amount of purified truncated selmet labeled polypeptide, prepared and purified as described in the Exemplification (mg/L of culture).	90.6		
Amount of purified, truncated selmet labeled polypeptide soluble in buffer, as described in the Exemplification (mg/ml of buffer)	64.7		
Minimum sequence coverage in the peptide fingerprint mapping analysis of truncated polypeptide, determined as described in EXAMPLE 9	45%	62%	60%
Z-score for the peptide fingerprint mapping analysis of truncated polypeptide, determined as described in EXAMPLE 9	1.9E-04	6.8E-06	7.4E-05
Number of matched peptides in the peptide fingerprint mapping analysis of truncated polypeptide, determined as described in EXAMPLE 9	12	15	13
Calculated molecular weight of truncated polypeptide (Da), determined as described in EXAMPLE 10	27530	27731	27958
Experimental molecular weight of truncated polypeptide (Da), determined as described in EXAMPLE 10	27685	27865	28109

The truncated polypeptides so expressed and purified are His tagged and have the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.

**FIGURE 216-D****TABLE 44 continued: Truncation Polypeptides of uridylylate kinase from *S. pneumoniae***

Start of truncated polypeptide of SEQ ID NO: 250	N5	K7	K9
End of truncated polypeptide of SEQ ID NO: 250	N241	T237	N241
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approximately two-thirds	Approximately two-thirds	Approximately two-thirds
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	No discernable expression		
Amount of purified truncated polypeptide, prepared and purified as described in the Exemplification (mg/L of culture).	86	33.1	7.4
Amount of purified, truncated polypeptide soluble in buffer, as described in the Exemplification (mg/ml of buffer)	43	33.1	16
Amount of purified truncated selmet labeled polypeptide, prepared and purified as described in the Exemplification (mg/L of culture).	60.5	24.7	94.9
Amount of purified, truncated selmet labeled polypeptide soluble in buffer, as described in the Exemplification (mg/ml of buffer)	60.5	12.4	18.9
Minimum sequence coverage in the peptide fingerprint mapping analysis of truncated polypeptide, determined as described in EXAMPLE 9	32%(1)	48%	59%
Z-score for the peptide fingerprint mapping analysis of truncated polypeptide, determined as described in EXAMPLE 9	4.8E-04(1)	2.5E-06	1.1E-06
Number of matched peptides in the peptide fingerprint mapping analysis of truncated polypeptide, determined as described in EXAMPLE 9	11(1)	11	12
Calculated molecular weight of truncated polypeptide (Da), determined as described in EXAMPLE 10	27931	27047	27157
Experimental molecular weight of truncated polypeptide (Da), determined as described in EXAMPLE 10	26060	27185	27412

The truncated polypeptides so expressed and purified are His tagged and have the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.

(1) Tryptic peptide mass spectrum peak searching was performed using selmet labeled truncated polypeptide.

## FIGURE 216-E

TABLE 44 continued: Truncation Polypeptides of uridylate kinase from *S. pneumoniae*

Start of truncated polypeptide of SEQ ID NO: 250	M3	N5	N5	N5	N5
End of truncated polypeptide of SEQ ID NO: 250	T237	T237	V239	I243	I243
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one-third	Approachably two-thirds	Less than one-third	Approachably two-thirds	Approachably two-thirds
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	No discernable expression		No discernable expression		

Start of truncated polypeptide of SEQ ID NO: 250	K7	K7	K7	K9	K9
End of truncated polypeptide of SEQ ID NO: 250	V239	N241	I243	T237	T237
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one-third	Approaching one-third	Approaching one-third	Less than one-third	Less than one-third
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	No discernable expression	No discernable expression			

Start of truncated polypeptide of SEQ ID NO: 250	K9	K9	I11	K14	K14
End of truncated polypeptide of SEQ ID NO: 250	V239	I243	V239	N234	N241
Solubility of truncated polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approachably two-thirds	Approaching 100%	No discernable expression	Less than one-third	Approaching one-third



**FIGURE 216-F**TABLE 44 continued: Truncation Polypeptides of uridylylate kinase from *S. pneumoniae*

PCR primers and restriction enzymes used to prepare the truncated polypeptides of TABLE 44, and the deleted amino acid residues in them, are set forth in the following tables:

Start of truncated polypeptide	M1	M3	N5	K7
Residues deleted from N-terminus	N/A	VK	VKMA	VKMANP
Nucleic acid sequence of forward PCR primer	SEQ ID NO: 253 G CGGCGGCCCAT ATGAAAATGGC GAATCCCAAG	SEQ ID NO: 254 G CGGCGGCCCAT ATGGCGAATCC CAAGTATAAAC	SEQ ID NO: 255 G CGGCGGCCCAT ATGAATCCCAA GTATAAACGTA TTTTAATC	SEQ ID NO: 256 G CGGCGGCCCAT ATGAAGTATAA ACGTATTTTAAT C
Restriction enzyme for forward PCR primer	NdeI	NdeI	NdeI	NdeI

Start of truncated polypeptide	K9	II1	K14
Residues deleted from N-terminus	VKMANPKY	VKMANPKYKR	VKMANPKYKRILI
Nucleic acid sequence of forward PCR primer	SEQ ID NO: 257 GCGGCG GCCCATATGAAACGTAT TTTAATCAAGTTATC	SEQ ID NO: 258 GCGGCG GCCCATATGATTTTAATC AAGTTATCAGG	SEQ ID NO: 259 GCGGCG GCCCATATGAAAGTTATC AGGTGAAGCC
Restriction enzyme for forward PCR primer	NdeI	NdeI	NdeI

**FIGURE 216-G**TABLE 44 continued: Truncation Polypeptides of uridylylate kinase from *S. pneumoniae*

PCR primers and restriction enzymes used to prepare the truncated polypeptides of TABLE 44, and the deleted amino acid residues in them, are set forth in the following tables:

End of truncated polypeptide	E247	T237	V239
Residues deleted from C-terminus	N/A	TVSNNIEEKE	SNNIEEKE
Nucleic acid sequence of reverse PCR primer	SEQ ID NO: 260 GCGCGG ATCCTTCCCTTTCTTCGAT ATTATTIG	SEQ ID NO: 261 GCGCGG ATCCTGTCCGATATTTC ACCAAATAC	SEQ ID NO: 262 GCGCGG ATCCAACTGTTGTCCGA TATTTTCAC
Restriction enzyme for reverse PCR primer	BamHI	BamHI	BamHI

End of truncated polypeptide	N241	I243	N234
Residues deleted from C-terminus	NIEEKE	EEKE	IGTTVSNNIEEKE
Nucleic acid sequence of reverse PCR primer	SEQ ID NO: 263 GCGCGG ATCCATTGAAACTGTG TTCCGATATTTC	SEQ ID NO: 264 GCGCGG ATCCGATATTATTGAAA CTGTTGTC	SEQ ID NO: 265 GCGCGG ATCCATTTTCACCAAATA CGACACG
Restriction enzyme for reverse PCR primer	BamHI	BamHI	BamHI

A blank in any of the parts of TABLE 44 for the truncated polypeptide experiments indicates that the experiment was not completed.

FIGURE 217

TABLE 45 Bioinformatic Analyses of uridylate kinase from *S. pneumoniae*

TABLE 45 -- uridylate kinase from <i>S. pneumoniae</i> -- SEQ ID NO: 247-SEQ ID NO: 250	
COG Category	Nucleotide Transport and Metabolism
COG ID Number	COG0528
Is SEQ ID NO: 248 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 248	none
Source organism for closest PDB protein to SEQ ID NO: 248	N/A
e-value for closest PDB Protein to SEQ ID NO: 248	N/A
% Identity between SEQ ID NO: 248 and the closest protein from PDB	N/A
% Positives between SEQ ID NO: 248 and the closest protein from PDB	N/A
Number of Protein Hits in the VGDB to SEQ ID NO: 248	12
Number of Microorganisms having VGDB Hits to SEQ ID NO: 248	12
Microorganisms having VGDB Hits to SEQ ID NO: 248 <sup>1</sup>	[efae][spne][saur][hinf][ecoli][paer][bsub][rpxx][nmen][ctra][hpyl][mgen]
First predicted epitopic region of SEQ ID NO: 248: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 266 :GIDIQTVQTIAKE-IQEVHSLGIEIALVIGG, 1.143,27->56
Second predicted epitopic region of SEQ ID NO: 248: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 267 :IDLVVFN, 1.142,213->219
Third predicted epitopic region of SEQ ID NO: 248: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 268 :VMNALVMADSLQ-QVGVDTRVQTALAMQQVAEPYVRGR, 1.133,84->120

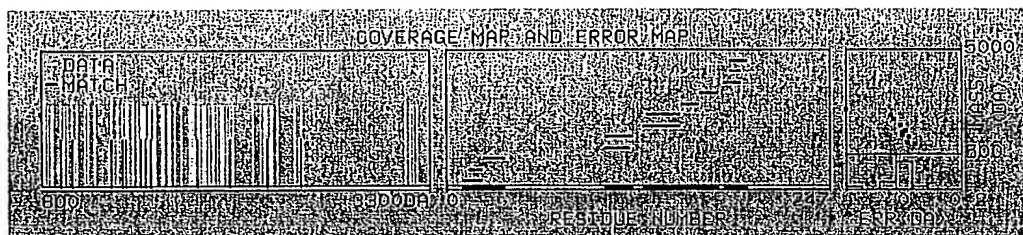
- <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Eschericia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpxx* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

FIGURE 218

Measured peptides: 79

Matched peptides: 12

Min. sequence coverage: 45%



Measured	Avg/	Computed	Error	Residues Missed			Peptide sequence
Mass(M)	Mono	Mass	(Da)	Start	To	Cut	
930.496	M	930.456	0.040	184	190	0	FEELTHR
1001.529	M	1001.514	0.015	15	24	0	LSGEALAGER
1247.596	M	1247.578	0.019	165	176	0	NGVDGVYNADPK
1329.702	M	1329.703	-0.002	180	190	1	TAVKFEELTHR
1344.694	M	1344.695	-0.001	152	164	0	AAEIEADAILMAK
1441.813	M	1441.813	-0.000	25	38	0	GVGIDIQTVQTIK
1468.860	M	1468.860	-0.000	11	24	1	ILIKLSGEALAGER
1499.783	M	1499.773	0.010	184	195	1	FEELTHRDVINK
1802.924	M	1802.934	-0.010	103	118	0	VQTAIAMQQVAEPYVR
2016.029	M	2016.057	-0.027	103	120	1	VQTAIAMQQVAEPYVRGR
2256.138	M	2256.178	-0.040	130	151	0	IVIFGAGIGSPYFSTDTTAALR
2469.111	M	2469.300	-0.189	128	151	1	GRIVIFGAGIGSPYFSTDTTAALR

FIGURE 219

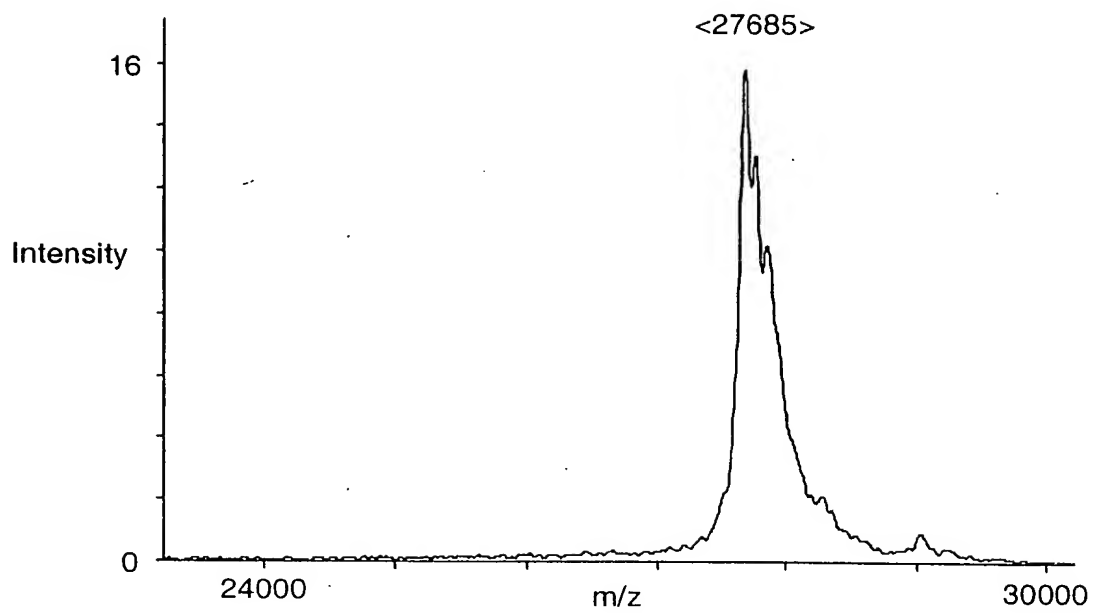
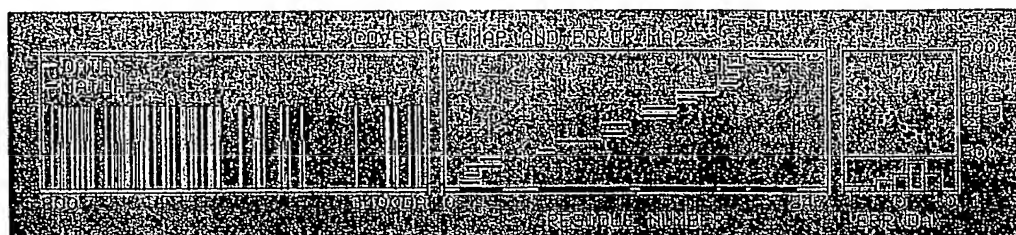


FIGURE 220

Measured peptides: 67

Matched peptides: 15

Min. sequence coverage: 62%

Note: click on the ☐ symbol to change column format.

Measured	Avg/	Computed	Error	<input type="checkbox"/> Residues	Missed		
Mass(M)	Mono	Mass	(Da)	Start	To	Cut	Peptide sequence
930.545	M	930.456	0.089	184	190	0	FEELTHR
1001.602	M	1001.514	0.088	15	24	0	LSGEALAGER
1102.527	M	1102.471	0.057	62	72	0	GEPAAEAGMDR
1329.725	M	1329.703	0.022	180	190	1	TAVKFEELTHR
1344.745	M	1344.695	0.049	152	164	0	AAEIEADAILMAK
1441.850	M	1441.813	0.037	25	38	0	GVGIDIQTVQTIK
1468.874	M	1468.860	0.014	11	24	1	ILIKLSGEALAGER
1499.801	M	1499.773	0.028	184	195	1	FEELTHRDVINK
1802.944	M	1802.934	0.010	103	118	0	VQTAIAMQQVAEPYVR
2016.008	M	2016.057	-0.048	103	120	1	VQTAIAMQQVAEPYVRGR
2256.147	M	2256.178	-0.031	130	151	0	IVIFGAGIGSPYFSTDTTAALR
2425.298	M	2425.316	-0.019	15	38	1	LSGEALAGERGVGIDIQTVQTIK
2469.180	M	2469.300	-0.120	128	151	1	GRIVIFGAGIGSPYFSTDTTAALR
2574.345	M	2574.262	0.083	152	176	1	AAEIEADAILMAKNGVDGVYNADPK
3182.392	M	3182.492	-0.100	199	227	0	IMDSTASTLSMDNDIDLNVENMNQPCNIK
3182.392	M	3182.540	-0.148	73	102	0	VQADYTGMLGTYMNAIVMADSLQQVGVDTR

FIGURE 221

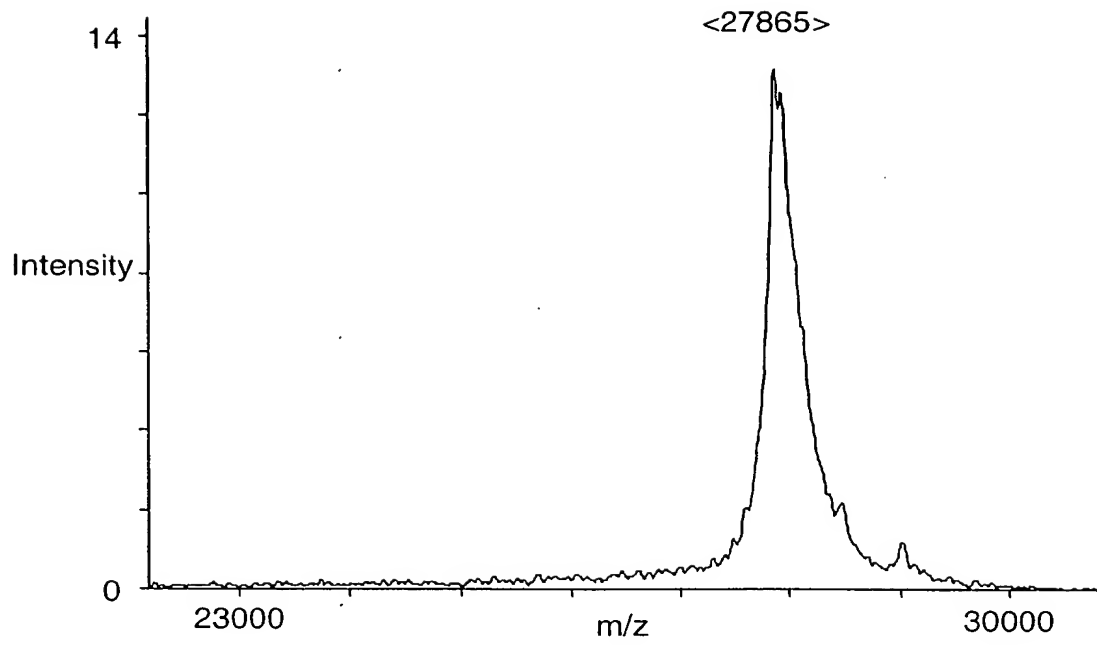
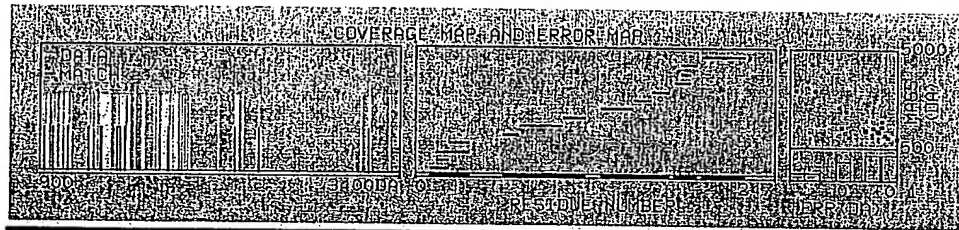


FIGURE 222

Measured peptides: 65  
 Matched peptides: 13  
 Min. sequence coverage: 60%



Measured Avg/	Computed	Error	Residues	Missed	
Mass(M) Mono	Mass	(Da)	Start To	Cut	Peptide sequence
930.586	M	930.456	0.131	184 190	0 FEELTHR
1001.624	M	1001.514	0.110	15 24	0 LSGEALAGER
1102.575	M	1102.471	0.104	62 72	0 GEPAAEAGMDR
1247.668	M	1247.578	0.090	165 176	0 NGVDGVYNADPK
1329.788	M	1329.703	0.084	180 190	1 TAVKFEELTHR
1344.752	M	1344.695	0.057	152 164	0 AAIEADAILMAK
1441.886	M	1441.813	0.073	25 38	0 GVGIDIQTVQTIK
1468.858	M	1468.860	-0.003	11 24	1 ILIKLSGEALAGER
1499.869	M	1499.773	0.096	184 195	1 FEELTHRDVINK
1803.030	M	1802.934	0.096	103 118	0 VQTAIAMQQVAEPYVR
2256.187	M	2256.178	0.009	130 151	0 IVIFGAGIGSPYFSTDTTAALR
2425.307	M	2425.316	-0.010	15 38	1 LSGEALAGERGVGIDIQTVQTIK
3182.427	M	3182.492	-0.065	199 227	0 IMDSTASTLSMDNDIDLVFENMNQPGNIK
3182.427	M	3182.540	-0.113	73 102	0 VQADYTGMLGTVMNALVMADSLQQVGVDTR



FIGURE 223

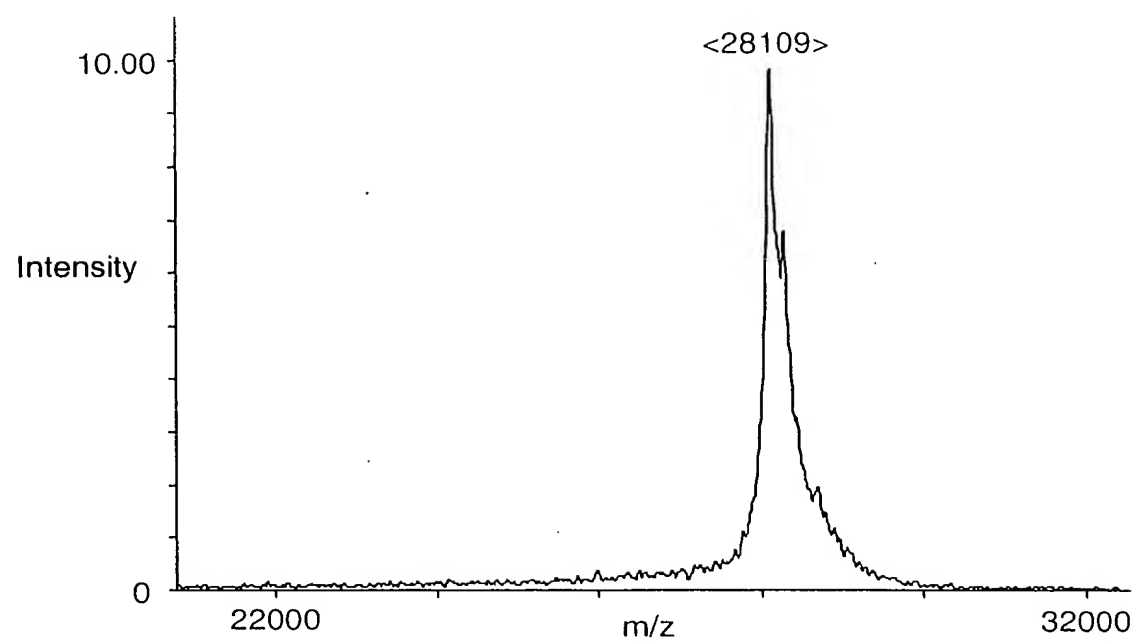


FIGURE 224

Measured peptides: 56

Matched peptides: 11

Min. sequence coverage: 32%



Measured Avg/ Computed	Error	Residues	Missed				
Mass(M) Mono. Mass	(Da)	Start To	Cut	Peptide sequence			
930.463 M 930.456	0.007	184 190	0	FEELTHR			
1001.514 M 1001.514	0.001	15 24	0	LSGEALAGER			
1102.481 M 1102.471	0.010	62 72	0	GEPAAEAGMDR			
1102.481 M 1102.471	0.010	62 72	0	GEPAAEAGMDR			
				(1)+c4@M;			
1247.531 M 1247.578	-0.047	165 176	0	NGVDGVYNADPK			
1329.721 M 1329.703	0.017	180 190	1	TAVKFEELTHR			
1344.687 M 1344.695	-0.009	152 164	0	AAEIEADAILMAK			
				(1)+c4@M;			
1344.687 M 1344.695	-0.009	152 164	0	AAEIEADAILMAK			
1441.786 M 1441.813	-0.028	25 38	0	GVGIDIQTVQTIK			
1468.867 M 1468.860	0.006	11 24	1	ILIKLSGEALAGER			
1499.761 M 1499.773	-0.012	184 195	1	FEELTHRDVINK			
1802.957 M 1802.934	0.023	103 118	0	VQTAIAMQQVAEPYVR			
1802.957 M 1802.934	0.023	103 118	0	VQTAIAMQQVAEPYVR			
				(1)+c4@M;			
2256.146 M 2256.178	-0.032	130 151	0	IVIFGAGIGSPYFSTDTTAALR			

FIGURE 225

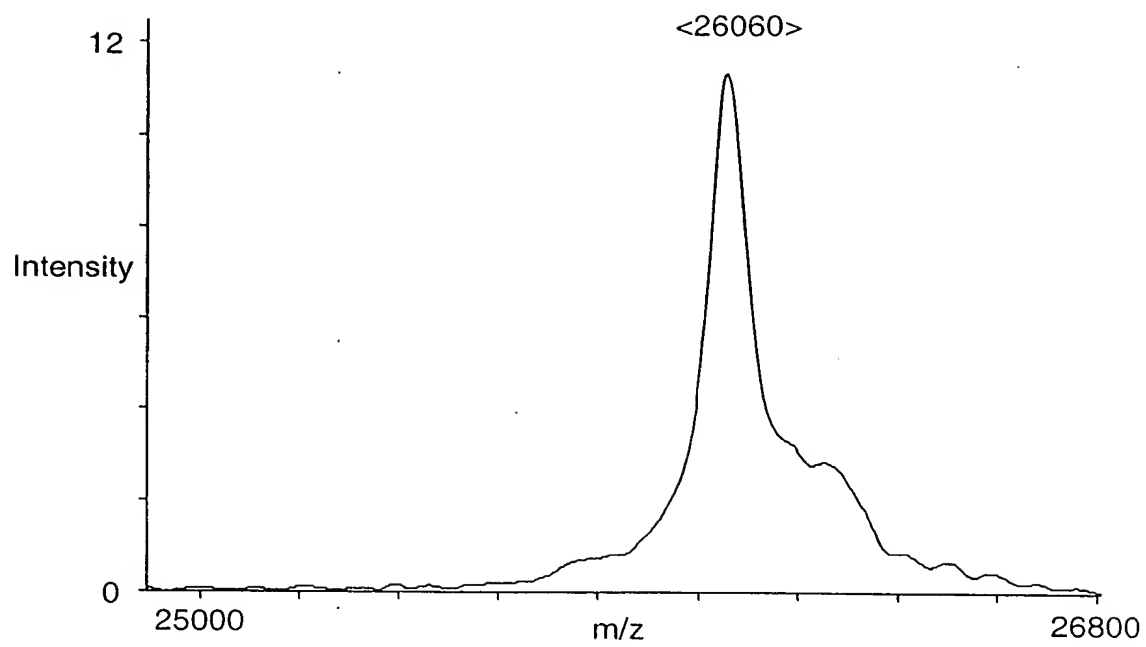
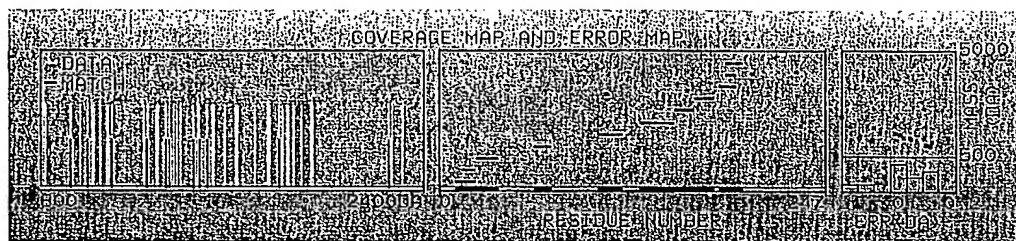


FIGURE 226

Measured peptides: 46

Matched peptides: 11

Min. sequence coverage: 48%



Measured	Avg/	Computed	Error	Residues		Missed	
Mass(M)	Mono	Mass	(Da)	Start	To	Cut	Peptide sequence
930.562	M	930.456	0.107	184	190	0	FEELTHR
1001.607	M	1001.514	0.094	15	24	0	LSGEALAGER
1102.586	M	1102.471	0.116	62	72	0	GEPAAEAGMDR
1247.623	M	1247.578	0.046	165	176	0	NGVDGVYNADPK
1329.721	M	1329.703	0.018	180	190	1	TAVKFEELTHR
1344.736	M	1344.695	0.041	152	164	0	AAEIADAILMAK
1441.823	M	1441.813	0.009	25	38	0	GVGIDIQTVQTIK
1468.870	M	1468.860	0.010	11	24	1	ILIKLSGEALAGER
1499.764	M	1499.773	-0.009	184	195	1	FEELTHRDVINK
1802.850	M	1802.934	-0.084	103	118	0	VQTAIAMQQAEPYVR
2255.986	M	2256.178	-0.192	130	151	0	IVIFGAGIGSPYFSTDTAALR

FIGURE 227

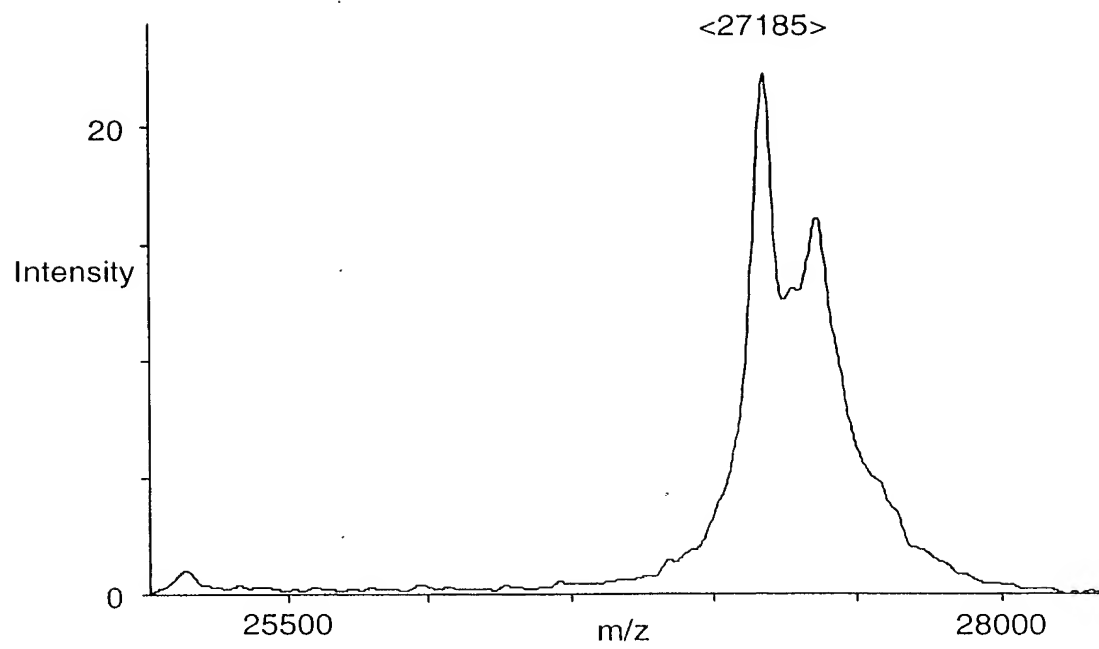
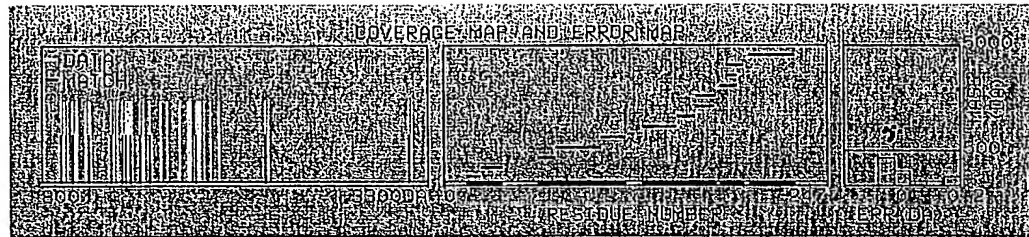


FIGURE 228

Measured peptides: 43

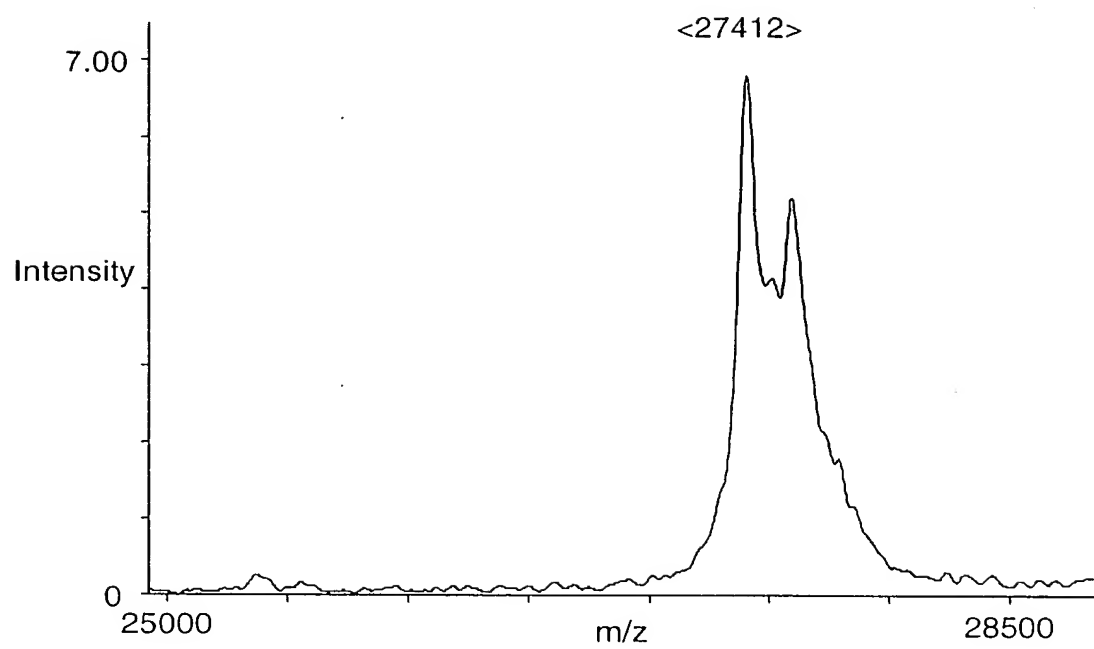
Matched peptides: 12

Min. sequence coverage: 59%



Measured	Avg/	Computed	Error	Residues		Missed	
Mass(M)	Mono	Mass	(Da)	Start	To	Cut	Peptide sequence
930.415	M	930.456	-0.041	184	190	0	FEELTHR
1001.480	M	1001.514	-0.034	15	24	0	LSGEALAGER
1102.441	M	1102.471	-0.029	62	72	0	GEPAEAGMDR
1247.549	M	1247.578	-0.028	165	176	0	NGVDGVYNADPK
1329.703	M	1329.703	-0.001	180	190	1	TAVKFEELTHR
1344.640	M	1344.695	-0.056	152	164	0	AAEIEADAILMAK
1375.629	M	1375.673	-0.044	165	177	1	NGVDGVYNADPKK
1441.762	M	1441.813	-0.051	25	38	0	GVGIDIQTVQTIK
1499.750	M	1499.773	-0.023	184	195	1	FEELTHRDVINK
1802.982	M	1802.934	0.048	103	118	0	VQTAIAMQQVAEPYVR
2256.273	M	2256.178	0.095	130	151	0	IVIFGAGIGSPYFSTDTTAALR
3182.691	M	3182.492	0.199	199	227	0	IMDSTASTLSMDNDIDLVFNMNQPGNIK
3182.691	M	3182.540	0.151	73	102	0	VQADYTGMLGTVMNALVMADSLQQVGVDTR

FIGURE 229



**FIGURE 230**

SEQ ID NO: 269

ATGGCTCAGCAACTGAGCGCTCGTCAACCTCGCTATAAACGCATTCTTCT  
5 AAAGTTGAGCGGCGAAGCCCTGATGGGCTCGGAGGAGTTCGGCATTGATCCCA  
AGGTGCTGGACCGCATGGCGCTGGAAATCGGCCAGTTGGTCGGGATCGGGCGTG  
CAGGTCGGCCTGGTCATCGGCGGGCGGCAACCTGTTCCGCGGGCGGGCCCTGTCC  
GCGGCCGGCATGGACCGGGTGACCGGGCGACCACATGGGGATGCTGGCCACCGT  
GATGAACGGCCTGGCGATGCGCGATGCGCTGGAGCGCTCGAACATCCCCGCGC  
10 TGGTGATGTCGGCGATCTCCATGGTCGGTGTGACCGACCACTACGACCGCCGCA  
AGGCCATGCGCCACCTCGGCGGTGGCGAGGTGGTGATCTTCTCCGCCGGTACCG  
GCAACCCGTTCTTCACCACCGACTCGGCGGGCTTGCCTGCGCGCCATCGAGATCG  
ACGCCGACGTGGTCCTTAAGGCTACCAAGGTCGATGGCGTGTACACTGCCGAC  
CCGTTCAAGGACCCGAATGCCGAGAAGTTCGAGCGCCTGACCTATGATGAAGT  
15 GCTCGACCGCAAGCTCGGCGTGATGGACCTGACCGCCATCTGCCTGTGCCGTGA  
CCAGAACATGCCGCTGCGGGTGTTCAACATGAACAAGCCGGGCGCATTGCTGA  
ATATTGTTGTTGGTGGTGCCGAAGGCACCCTGATCGAGGAGGGTTGA



**FIGURE 231**

SEQ ID NO: 270

MAQQLSARQPRYKRILLKLSGEALMGSEEFGIDPKVLDRMALEIGQLVGIG  
5 VQVGLVIGGGNLFRGAALSAAGMDRVTGDHMGMLATVMNGLAMRDALERSNIP  
ALVMSAISMVGVTGDHYDRRKAMRHLGGGEVVIFSAGTGNPFFTTDSAACLRAIEID  
ADVVLKATKVDGVYTADPFKDPNAEKFERLTIDEVLDRKLGVMDLTAICLCRDQ  
NMPLRVFNMNKPGALLNIVVGGAEGTLIEEG

**FIGURE 232**

SEQ ID NO: 271

ATGGCTCAGCAACTGAGCGCTCGTCAACCTCGCTATAAACGCATTCTTCT  
5 AAAGTTGAGCGGCGAAGCCCTGATGGGCTCGGAGGAGTTCGGCATCGATCCCA  
AGGTGCTGGACCGCATGGCGCTGGAAATCGGCCAGTTGGTCGGGATCGGCGTG  
CAGGTCGGCCTGGTCATCGGCGGCGGCAACCTGTTCCGCGGCGCGGCCCTGTCC  
GCGGCCGGCATGGACCGGGTGACCGGCGACCACATGGGGATGCTGGCCACCGT  
GATGAACGGCCTGGCGATGCGCGATGCGCTGGAGCGCTCGAACATCCCCGCGC  
10 TGGTGATGTCGGCGATCTCCATGGTCGGTGTGACCGACCACTACGACCGCCGCA  
AGGCCATGCGCCACCTCGGCGGTGGCGAGGTGGTGATCTTCTCCGCCGGTACCG  
GCAACCCGTTCTTCACCACCGACTCGGCGGCTTGCCTGCGCGCCATCGAGATCG  
ACGCCGACGTGGTCCTTAAGGCTACCAAGGTCGATGGCGTGTACACTGCCGAC  
CCGTTCAAGGACCCGAATGCCGAGAAGTTCGAGCGCCTGACCTATGATGAAGT  
15 GCTCGACCGCAAGCTCGGCGTGATGGACCTGACCGCCATCTGCCTGTGCCGTGA  
CCAGAACATGCCGCTGCGGGTGTTCAACATGAACAAGCCGGGCGCATTGCTGA  
ATATTGTTGTTGGTGGTGCCGAAGGCACCCTGATCGAGGAGGGTTGA

**FIGURE 233**

SEQ ID NO: 272

MAQQLSARQPRYKRILLKLSGEALMGSEEFGIDPKVLDRMALEIGQLVGIG  
5 VQVGLVIGGGNLFRGAALSAAGMDRVTGDHMGMLATVMNGLAMRDALERSNIP  
ALVMSAISMVGVTDHYDRRKAMRHLGGGEVVIFSAGTGNPFFTTDSAACLRAIEID  
ADVVLKATKVDGVYTADPFKDPNAEKFERLTYDEVLDRLGVMDLTAICLCRDQ  
NMPLRVFNMNKP GALLNIVVGGAEGTLIEEG

**FIGURE 234**

SEQ ID NO: 273

Forward PCR Primer

5 GCGGCGGCCCATATGGCTCAGCAACTGAGCG

SEQ ID NO: 274

10

GCGCGGATCCACCCTCCTCGATCAGGGTG

**FIGURE 235****TABLE 46 Properties of uridylate kinase from *P. aeruginosa***

TABLE 46 -- uridylate kinase from <i>P. aeruginosa</i> -- SEQ ID NO: 269-SEQ ID NO: 272	
Melting temperature (°C) of SEQ ID NO: 273 (forward PCR primer)	60
Restriction enzyme for SEQ ID NO: 273 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 274 (reverse PCR primer)	62
Restriction enzyme for SEQ ID NO: 274 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 269	738
Number of amino acid residues in SEQ ID NO: 270	245
Number of different nucleic acid residues between SEQ ID NO: 269 and SEQ ID NO: 271	1
Number of different amino acid residues between SEQ ID NO: 270 and SEQ ID NO: 272	0
Calculated molecular weight of SEQ ID NO: 270 polypeptide (kDa)	26.3
Calculated pI of SEQ ID NO: 270 polypeptide	5.5
Solubility of SEQ ID NO: 272 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one-third
Solubility of SEQ ID NO: 272 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	Approaching 100%
Amount of purified polypeptide having SEQ ID NO: 272, prepared and purified as described in the Exemplification (mg/mL of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	45.1
Amount of purified polypeptide having SEQ ID NO: 272 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	112.8

**FIGURE 235-B**

TABLE 46 -- uridylate kinase from <i>P. aeruginosa</i> -- SEQ ID NO: 269-SEQ ID NO: 272	
Z-score for the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 272, determined as described in EXAMPLE 9	3.7E-3
Number of matched peptides in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 272, determined as described in EXAMPLE 9	8
Minimum sequence coverage in the peptide fingerprint mapping analysis of polypeptide having SEQ ID NO: 272, determined as described in EXAMPLE 9	39
Calculated molecular weight of SEQ ID NO: 270 polypeptide (Da), determined as described in EXAMPLE 10	28304
Experimental molecular weight of SEQ ID NO: 272 polypeptide (Da), determined as described in EXAMPLE 10	28459
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. The identity of an interacting protein identified by using at least one of the methods described in those examples is: DnaK protein (gi 9951024).	
Crystals of a polypeptide having the sequence of SEQ ID NO: 272, prepared and purified as described above and having a His tag, are obtained using the following conditions: 1.6M ammonium sulfate, 0.1M HEPES pH 7.5, 0.1M NaCl. The crystals were prepared using the following method: 20°C, sitting-drop, 15 mg polypeptide per ml of solution.	

## FIGURE 236

TABLE 47 Bioinformatic Analyses of uridylate kinase from *P. aeruginosa*

TABLE 47 -- uridylate kinase from <i>P. aeruginosa</i> -- SEQ ID NO: 269-SEQ ID NO: 272	
COG Category	Nucleotide Transport and Metabolism
COG ID Number	COG0528
Is SEQ ID NO: 270 classified as an essential gene?	yes
Most closely related protein from PDB to SEQ ID NO: 270	none
Source organism for closest PDB protein to SEQ ID NO: 270	N/A
e-value for closest PDB Protein to SEQ ID NO: 270	N/A
% Identity between SEQ ID NO: 270 and the closest protein from PDB	N/A
% Positives between SEQ ID NO: 270 and the closest protein from PDB	N/A
Number of Protein Hits in the VGDB to SEQ ID NO: 270	12
Number of Microorganisms having VGDB Hits to SEQ ID NO: 270	12
Microorganisms having VGDB Hits to SEQ ID NO: 270 <sup>1</sup>	[paer][efae][saur][hinf][ecoli][rpxx][spne][nmen][bsub][hpyl][ctra][mgen]
First predicted epitopic region of SEQ ID NO: 270: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 275 :YDEVLDRLKLGVM-DLTAICLCRD, 1.207,192->213
Second predicted epitopic region of SEQ ID NO: 270: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 276 :EIGQLVGIGVQVGLVIGG, 1.206,43->60,
Third predicted epitopic region of SEQ ID NO: 270: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 277 :GALLNIVVGG, 1.180,227->236

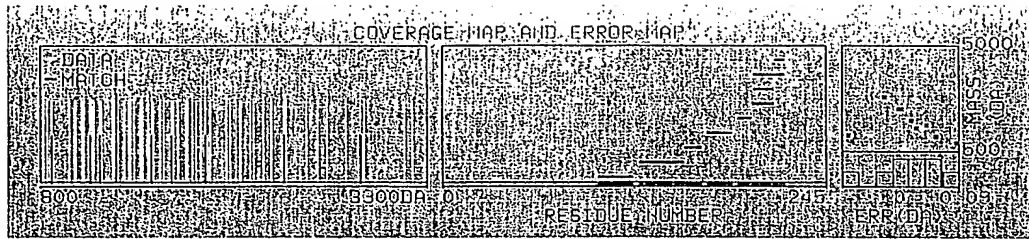
- <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Eschericia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpxx* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

FIGURE 237

Measured peptides: 58

Matched peptides: 8

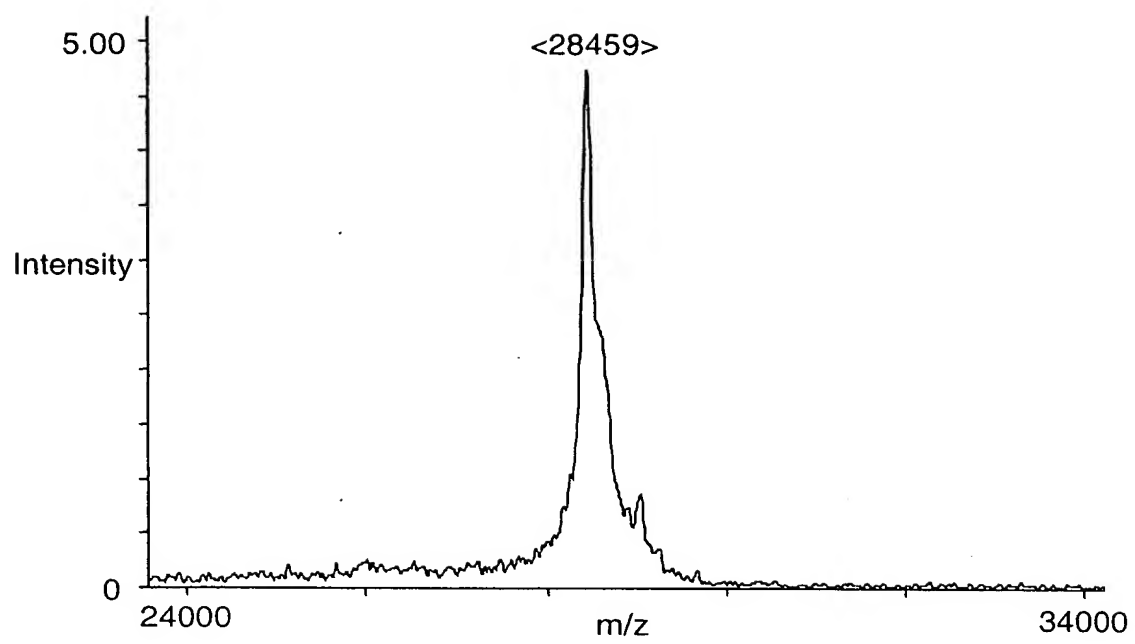
Min. sequence coverage: 39%



Measured	Avg/	Computed	Error	Residues		Missed	
Mass(M)	Mono	Mass	(Da)	Start	To	Cut	Peptide sequence
872.509	M	872.417	0.092	213	219	0	DQNMPLR
1122.613	M	1122.555	0.058	190	198	0	LTYDEVLDR
1184.585	M	1184.665	-0.080	156	166	0	AIEIDADVVLK
1520.776	M	1520.750	0.026	200	212	0	LGVMDLTAICLCR
1648.888	M	1648.845	0.043	199	212	1	KLGVMDLTAICLCR
1864.902	M	1864.884	0.019	170	186	1	VDGVYTADPFKDPNAEK
2375.159	M	2375.157	0.003	200	219	1	LGVMDLTAICLCRDQNMPLR
2375.159	M	2375.160	-0.001	101	122	0	SNIPALVMSAISMGVTDHYDR
2880.362	M	2880.385	-0.023	128	155	0	HLGGGEVVIFSAGTGNPFFTTDSAACLR



FIGURE 238



**FIGURE 239**

SEQ ID NO: 278

ATGGCTAAAAAAATTGTTTCTGATTTAGATCTTAAAGGTAAAACAGTCCT  
5 AGTACGTGCTGATTTTAAACGTACCTTTAAAAGACGGTGAAATTACTAATGACAA  
CCGTATCGTTCAAGCTTTACCTACAATTCAATACATCATCGAACAAGGTGGTAA  
AATCGTACTATTTTCACATTTAGGTAAAGTGAAAGAAGAAAGTGATAAAGCAA  
AATTAACTTTACGTCCAGTTGCTGAAGACTTATCTAAGAAATTAGATAAAGAAG  
TTGTTTTCTGACCAGAAACACGCGGCGAAAACTTGAAGCTGCTATTAAAGACC  
10 TTAAAGAAGGCGACGTATTATTAGTTGAAAATACACGTTATGAAGATTTAGAC  
GGTAAAAAAGAATCTAAAAATGATCCAGAATTAGGTAAATACTGGGCATCTTT  
AGGTGATGTGTTTGTAAATGATGCTTTTGGTACTGCGCATCGTGAGCATGCATC  
TAATGTTGGTATTTCTACACATTTAGAAACTGCAGCTGGATTCTTAATGGATAA  
AGAAATTAAGTTTATTGGCGGCGTAGTTAACGATCCACATAAACCAGTTGTTGC  
15 TATTTTAGGTGGAGCAAAAGTATCTGACAAAATTAATGTCATCAAAAACCTTAGT  
TAACATAGCTGATAAAATTATCATCGGCGGAGGTATGGCTTATACTTTCTTAAA  
AGCGCAAGGTAAAGAAATTGGTATTTCAATTATTAGAAGAAGATAAAATCGACT  
TCGCAAAAGATTTATTAGAAAAACATGGTGATAAAATTGTATTACCAGTAGAC  
ACTAAAGTTGCTAAAGAATTTTCTAATGATGCCAAAATCACTGTAGTACCATCT  
20 GATTCAATTCCAGCAGACCAAGAAGGTATGGATATTGGACCAAAACACTGTAAA  
ATTATTTGCAGATGAATTAGAAGGTGCGCACACTGTTGTATGGAATGGACCTAT  
GGGTGTATTCGAGTTCAGTAACTTTGCACAAGGTACAATTGGTGTATGTAAAGC  
AATTGCAAACCTTAAAGATGCAATTACGATTATCGGTGGCGGTGATTCAGCTGC  
AGCAGCAATCTCTTTAGGTTTTGAAAATGACTTCACTCATATTTCAACTGGTGG  
25 CGGCGCGTCATTAGAGTACCTAGAAGGTAAAGAATTGCCTGGTATCAAAGCAA  
TCAATAATAAATAA

**FIGURE 240**

SEQ ID NO: 279

MAKKIVSDLDLKGKTVLVRADFNVPLKDGEITNDNRIVQALPTIQYIIEQGG  
5 KIVLFSHLGKVKEESDKAKLTLRPVAEDLSKKLDKEVVFPETRGEKLEAAIKDLK  
EGDVLLVENTRYEDLDGKKESKNDPELGKYWASLGDVVFVNDAFGTAHREHASNV  
GISTHLETAAGFLMDKEIKFIGGVVNDPHKPVVAILGGAKVSDKINVIKNLVNIADK  
IIGGGMAYTFLKAQGKEIGISLLEEDKIDFAKDLLEKHGDKIVLPVDTKVAKEFSND  
AKITVVPDSIPADQEGMDIGPNTVKLFADELEGAHTVVWNGPMGVFEFSNFAQGT  
10 IGVCKAIANLKDAITIIGGGDSAAAAISLGFENDFTHISTGGGASLEYLEGKELPGIK  
AINNK

**FIGURE 241**

SEQ ID NO: 280

ATGGCTAAAAAAATTGTTTCTGATTTAGATCTTAAAGGTAAAACAGTCCT  
5 AGTACGTGCTGATTTTAAACGTACCTTTAAAAGACGGTGAAATTACTAATGACAA  
CCGTATCGTTCAAGCTTTACCTACAATTCAATACATCATCGAACAAGGTGGTAA  
AATCGTACTATTTTCACATTTAGGTAAAGTGAAAGAAGAAAGTGATAAAGCAA  
AATTAACTTTACGTCCAGTTGCTGAAGACTTATCTAAGAAATTAGATAAAGAAG  
TTGTTTTTCGTACCAGAAACACGCGGCGAAAACTTGAAGCTGCTATTAAAGACC  
10 TTAAAGAAGGCGACGTATTATTAGTTGAAAATACACGTTATGAAGATTTAGAC  
GGTAAAAAAGAATCTAAAAATGATCCAGAATTAGGTAAATACTGGGCATCTTT  
AGGTGATGTGTTTGTAATGATGCTTTTGGTACTGCGCATCGTGAGCATGCATC  
TAATGTTGGTATTTCTACACATTTAGAAACTGCAGCTGGATTCTTAATGGATAA  
AGAAATTAAGTTTATTGGCGGCGTAGTTAACGATCCACATAAACCAGTTGTTGC  
15 TATTTTAGGTGGAGCAAAAGTATCTGACAAAATTAATGTCATCAAAAACCTTAGT  
TAACATAGCTGATAAAATTATCATCGGCGGAGGTATGGCTTATACTTTCTTAAA  
AGCGCAAGGTAAAGAAATTGGTATTTCAATTATTAGAAGAAGATAAAATCGACT  
TCGCAAAAGATTTATTAGAAAAACATGGTGATAAAATTGTATTACCAGTAGAC  
ACTAAAGTTGCTAAAGAATTTTCTAATGATGCCAAAATCACTGTAGTACCATCT  
20 GATTCAATTCCAGCAGACCAAAAAGGTATGGATATTGGACCAAACACTGTAAA  
ATTATTTGCAGATGAATTAGAAGGTGCGCACACTGTTGTATGGAATGGACCTAT  
GGGTGTATTCGAGTTCAGTAACTTTGCACAAGGTACAATTGGTGTATGTAAAGC  
AATTGCAAACCTTAAAGATGCAATTACGATTATCGGTGGCGGTGATTCAGCTGC  
AGCAGCAATCTCTTTAGGTTTTGAAAATGACTTCACTCATATTTCAACTGGTGG  
25 CGGCGCGTCATTAGAGTACCTAGAAGGTAAAGAATTGCCTGGTATCAAAGCAA  
TCAATAATAAATAA

**FIGURE 242**

SEQ ID NO: 281

MAKKIVSDLDLKGKTVLVRADFNVPLKDGEITNDNRIVQALPTIQYIIEQGG  
5 KIVLFSHLGKVKEESDKAKLTLRPVAEDLSKKLDKEVVFPETRGEKLEAAIKDLK  
EGDVLLVENTRYEDLDGKKESKNDPELGKYWASLGDVVFVNDAFGTAHREHASNV  
GISTHLETAAGFLMDKEIKFIGGVVNDPHKPVVAILGGAKVSDKINVIKNLVNIADK  
IIIGGGMAYTFLKAQGKEIGISLLEEDKIDFAKDLLEKHGDKIVLPVDTKVAKDFSND  
AKITVVPSDSIPADQKGMDIGPNTVKLFADELEGAHTVVWNGPMGVFEFSNFAQG  
10 TIGVCKAIAANLKDAITIIGGGDSAAAAISLGFENDFTHISTGGGASLEYLEGKELPGIK  
AINNK

**FIGURE 243**

SEQ ID NO: 282

Forward PCR Primer

5 GCGGCGGCCCATATGGCTAAAAAAATTGTTTCTGATTAG

SEQ ID NO: 283

10

Reverse PCR Primer

GCGCGGATCCTTTCTTAGATAAGTCTTCAGCAAC

**FIGURE 244****TABLE 48 Properties of phosphoglycerate kinase from *S. aureus***

TABLE 48 -- phosphoglycerate kinase from <i>S. aureus</i> -- SEQ ID NO: 278-SEQ ID NO: 281	
Melting temperature (°C) of SEQ ID NO: 282 (forward PCR primer)	70
Restriction enzyme for SEQ ID NO: 282 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 283 (reverse PCR primer)	64
Restriction enzyme for SEQ ID NO: 283 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 278	1191
Number of amino acid residues in SEQ ID NO: 279	396
Number of different nucleic acid residues between SEQ ID NO: 278 and SEQ ID NO: 280	1
Number of different amino acid residues between SEQ ID NO: 279 and SEQ ID NO: 281	1
Calculated molecular weight of SEQ ID NO: 279 polypeptide (kDa)	42.6
Calculated pI of SEQ ID NO: 279 polypeptide	4.9
Solubility of SEQ ID NO: 281 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching 100%
Solubility of SEQ ID NO: 281 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	No detectable expression
Amount of purified polypeptide having SEQ ID NO: 281, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	10.74
Amount of purified polypeptide having SEQ ID NO: 281 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	35.80
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. The identity of an interacting proteins identified by using at least one of the methods described in those examples is: glyceraldehyde-3-phosphate dehydrogenase (gi 13700663)	

## FIGURE 245

TABLE 49 Bioinformatic Analyses of phosphoglycerate kinase from *S. aureus*

TABLE 49 -- phosphoglycerate kinase from <i>S. aureus</i> -- SEQ ID NO: 278-SEQ ID NO: 281	
COG Category	carbohydrate transport and metabolism
COG ID Number	COG0126
Is SEQ ID NO: 279 classified as an essential gene?	Yes
Most closely related protein from PDB to SEQ ID NO: 279	phosphoglycerate kinase, (1vpe)
Source organism for closest PDB protein to SEQ ID NO: 279	<i>Thermotoga maritima</i>
e-value for closest PDB Protein to SEQ ID NO: 279	1E-123
% Identity between SEQ ID NO: 279 and the closest protein from PDB	57
% Positives between SEQ ID NO: 279 and the closest protein from PDB	72
Number of Protein Hits in the VGDB to SEQ ID NO: 279	12
Number of Microorganisms having VGDB Hits to SEQ ID NO: 279	12
Microorganisms having VGDB Hits to SEQ ID NO: 279 <sup>1</sup>	[efae][saur][bsub][spne][ctra][bbur][ecoli][hinf][paer][mgen][hpyl][nmen]
First predicted epitopic region of SEQ ID NO: 279: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 284 :GKIVLFSHLGKV, 1.178, 52->63
Second predicted epitopic region of SEQ ID NO: 279: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 285 :IGGVVNDPHKPV-VAILGGAKVSDKINVILNVLNIADKIII, 1.175, 183->222
Third predicted epitopic region of SEQ ID NO: 279: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 286 :DKEVVFVPE, 1.155, 86->94

- <sup>1</sup>Organisms are abbreviated as follows: ecoli = *Eschericia coli*; hpyl = *Helicobacter pylori*; paer = *Pseudomonas aeruginosa*; ctra = *Chlaydia trachomatis*; hinf = *Haemophilus influenzae*; nmen = *Neisseria meningitidis*; rpxx = *Rickettsia prowazekii*; bbur = *Borrelia burgdorferi*; bsub = *Bacillus subtilis*; staph = *Staphylococcus aureus*; spne = *Streptococcus pneumoniae*; mgen = *Mycoplasma genitalium*; efae = *Enterococcus faecalis*.



**FIGURE 246**

SEQ ID NO: 287

ATGAAGGCACGACAACAAAAGTATTGTGATAAAATCGCCAACTTCTGGT  
5 GTCACCCTACAGGAAAAATCATCATGAGCCTGGCCGGTAAAAAAATCGTTCTC  
GGCGTTAGCGGCGGTATTGCTGCCTATAAAACCCCTGAACTGGTGCCTCGTTTG  
CGCGATCGCGGGGCGACGTCCGCGTAGCCATGACCGAAGCGGCAAAAGCCTT  
TATCACCCCACTTAGCTTGCAGGCGGTTTCTGGTTATCCCGTTTCCGACAGTCTG  
CTGGACCCGGCAGCCGAAGCCGCTATGGGCCATATTGAGCTGGGTAAATGGGC  
10 TGATTTAGTGATTCTCGCCCCTGCCACGGCAGATTTGATTGCCCGTGTTGCTGCC  
GGAATGGCGAATGACCTGGTATCGACGATTTGTCTGGCTACACCTGCGCCTGTA  
GCCGTGCTCCCCGCCATGAACCAGCAGATGTACCGTGCCGCTGCCACGCAGCAT  
AATTTAGAGGTGCTTGCTTCCCGTGTTTGCTCATCTGGGGGCCAGACAGTGGC  
AGTCAGGCTTGTGGTGATATCGGTCCTGGGCGAATGCTCGATCCGTTAACCATT  
15 GTGGATATGGCGGTAGCGCATTTTTTCGCCCCGTCAACGACCTGAAACATCTGAAC  
ATTATGATTACCGCCGGCCCGACGCGTGAACCGCTCGATCCGGTGCGTTATATC  
TCTAATCACAGCTCCGGCAAGATGGGTTTTGCTATCGCCGCCGCCGCTGCCCCGT  
CGTGGCGCGAACGTACGCTGGTATCAGGTCCGGTTTCACTACCGACGCCACCG  
TTTGTTAAACGTGTTGATGTGATGACCGCGCTGGAAATGGAAGCCGCCGTGAAT  
20 GCTTCTGTACAGCAGCAAAATATTTTTATCGGCTGCGCCGCCGTGGCGGATTAT  
CGCGCAGCTACCGTGGCCCCAGAGAAAATCAAAAAGCAGGCCACGCAGGGTG  
ATGAATTAACAATAAAAATGGTTAAAAACCCCGATATCGTCGCAGGCGTTGCC  
GCACTAAAAGACCATCGACCCTACGTCGTTGGATTTGCCGCCGAAACAAATAA  
TGTGGAAGAATACGCCCCGGCAAAAACGTATCCGTAAAAACCTTGATCTGATCT  
25 GCGCGAACGATGTTTCCCAGCCAACTCAAGGATTTAACAGCGACAACAACGCA  
TTACACCTTTTCTGGCAGGACGGAGATAAAGTCTTACCGCTTGAGCGCAAAGAG  
CTCCTTGGCCAATTATTACTCGACGAGATCGTGACCCGTTATGATGAAAAAAAT  
CGACGTAA

**FIGURE 247**

SEQ ID NO: 288

          MKARQQKYCDKIANFWCHPTGKIIMSLAGKKIVLGVSGGIAAYKTPELVRR  
5  LRDRGADVVRVAMTEAAKAFITPLSLQAVSGYPVSDSLLDPAAEAAMGHIELGKWA  
      DLVILAPATADLIARVAAGMANDLVSTICLATPAPVAVLPAMNQMYRAAATQH  
      NLEVLASRGLLIWGPDSGSQACGDIGPGRMLDPLTIVDMAVAHFSPVNDLKHLNIM  
      ITAGPTREPLDPVRYISNHSSGKMFGAIAAAAARRGANVTLVSGPVS LPTPPFVKRV  
      DVMTALEMEAAVNASVQQQNIFIGCAAVADYRAATVAPEKIKKQATQGDELTIK  
10 MVKNPDIVAGVAALKDHRPYVVGFAAETNNVEEYARQKRIRKNLDLICANDVSQP  
      TQGFNSDNNALHLFWQDGDKVLPLERKELLGQLLLDEIVTRYDEKNRR

**FIGURE 248**

SEQ ID NO: 289

ATGAAGGCACGACAACAAAAGTATTGTGATAAAATCGCCAACTTCTGGT  
5 GTCACCCTACAGGAAAAATCATCATGAGCCTGGCCGGTAAAAAAATCGTTCTC  
GGCGTTAGCGGCGGTATTGCTGCCTATAAAACCCCTGAACTGGTGCCTCGTTTG  
CGCGATCGCGGGGCCGACGTCCGCGTAGCCATGACCGAAGCGGCAAAAGCCTT  
TATCACCCCACTTAGCTTGCAGGCGGTTTCTGGTTATCCCGTTTCCGACAGTCTG  
CTGGACCCGGCAGCCGAAGCCGCTATGGGCCATATTGAGCTGGGTAAATGGGC  
10 TGATTTAGTGATTCTCGCCCCTGCCACGGCAGATTTGATTGCCCGTGTTGCTGCC  
GGAATGGCGAATGACCTGGTATCGACGATTTGTCTGGCTACACCTGCGCCTGTA  
GCCGTGCTCCCCGCCATGAACCAGCAGATGTACCGTGCCGCTGCCACGCAGCAT  
AATTTAGAGGTGCTTGCTTCCCGTGTTTGCTCATCTGGGGGCCAGACAGTGGC  
AGTCAGGCTTGTGGTGATATCGGTCCTGGGCGAATGCTCGATCCGTTAACCATT  
15 GTGGATATGGCGGTAGCGCATTTTTTCGCCCCGTCAACGACCTGAAACATCTGAAC  
ATTATGATTACCGCCGGCCCGACGCGTGAACCGCTCGATCCGGTGCGTTATATC  
TCTAATCACAGCTCCGGCAAGATGGGTTTTGCTATCGCCGCCGCCGCTGCCCCGT  
CGTGGCGCGAACGTACGCTGGTATCAGGTCCGGTTTCACTACCGACGCCACCG  
TTTGTTAAACGTGTTGATGTGATGACCGCGCTGGAAATGGAAGCCGCCGTGAAT  
20 GCTTCTGTACAGCAGCAAAATATTTTTATCGGCTGCGCCGCCGTGGCGGATTAT  
CGCGCAGCTACCGTGGCCCCAGAGAAAAATCAAAAAGCAGGCCACGCAGGGTG  
ATGAATTAACAATAAAAATGGTTAAAAACCCCGATATCGTCGCAGGCGTTGCC  
GCACTAAAAGACCATCGACCCTACGTCGTTGGGTTTGCCGCCGAAACAAATAA  
TGTGGAAGAATACGCCCGGCAAAAACGTATCCGTAAAAACCTTGATCTGATCT  
25 GCGCGAACGATGTTTCCCAGCCAACTCAAGGATTAAACAGCGACAACAACGCA  
TTACACCTTTTCTGGCAGGACGGAGATAAAGTCTTACCGCTTGAGCGCAAAGAG  
CTCCTTGGCCAATTATTACTCGACGAGATCGTGACCCGTTATGATGAAAAAAT  
CGACGTAA

**FIGURE 249**

SEQ ID NO: 290

          MKARQQKYCDKIANFWCHPTGKIIMSLAGKKIVLGVSSGGIAAYKTPELVRR  
5  LRDRGADVVRVAMTEAAKAFITPLSLQAVSGYPVSDSLLDPAAEAAMGHIELGKWA  
      DLVILAPATADLIARVAAGMANDLVSTICLATPAPVAVLPAMNQMYRAAATQH  
      NLEVLASRGLLIWGPDSGSQACGDIGPGRMLDPLTIVDMAVAHFSPVNDLKHLNIM  
      ITAGPTREPLDPVRYISNHSSGKMGFAIAAAAAARRGANVTLVSGPVSLPTPPFVKRV  
      DVMTALEMEAAVNASVQQQNIFIGCAAVADYRAATVAPEKIKKQATQGDELTIK  
10  MVKNPDIVAGVAALKDHRPYVVGFAAETNNVEEYARQKRIRKNLDLICANDVSQP  
      TQGFNSDNNALHLFWQDGDKVLPLERKELLGQLLLDEIVTRYDEKNRR

**FIGURE 250**

SEQ ID NO: 291

Forward PCR Primer

5 GCGGCGGCCCATATGAAGGCACGACAACAAAAG

SEQ ID NO: 292

10

Reverse PCR Primer

GCGCGGATCCAACGGGATAACCAGAAACCG

**FIGURE 251****TABLE 50 Properties of flavoprotein affecting synthesis of DNA and pantothenate from *E. coli***

TABLE 50 -- flavoprotein affecting synthesis of DNA and pantothenate from <i>E. coli</i> -- SEQ ID NO: 287-SEQ ID NO: 290	
Melting temperature (°C) of SEQ ID NO: 291 (forward PCR primer)	60
Restriction enzyme for SEQ ID NO: 291 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 292 (reverse PCR primer)	60
Restriction enzyme for SEQ ID NO: 292 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 287	1294
Number of amino acid residues in SEQ ID NO: 288	430
Number of different nucleic acid residues between SEQ ID NO: 287 and SEQ ID NO: 289	0
Number of different amino acid residues between SEQ ID NO: 288 and SEQ ID NO: 290	0
Calculated molecular weight of SEQ ID NO: 288 polypeptide (kDa)	46.3
Calculated pI of SEQ ID NO: 288 polypeptide	8.2
Solubility of SEQ ID NO: 290 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching one-third
Solubility of SEQ ID NO: 290 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	No detectable expression
Amount of purified polypeptide having SEQ ID NO: 290, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	8.80
Amount of purified polypeptide having SEQ ID NO: 290 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	30.00
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. No interacting proteins were observed by using at least one of the methods described in those examples.	

**FIGURE 252****TABLE 51 Bioinformatic Analyses of flavoprotein affecting synthesis of DNA and pantothenate from *E. coli***

TABLE 51 -- flavoprotein affecting synthesis of DNA and pantothenate from <i>E. coli</i> -- SEQ ID NO: 287-SEQ ID NO: 290	
COG Category	coenzyme metabolism
COG ID Number	COG0452
Is SEQ ID NO: 288 classified as an essential gene?	Yes
Most closely related protein from PDB to SEQ ID NO: 288	halotolerance protein Hal3, (1e20)
Source organism for closest PDB protein to SEQ ID NO: 288	<i>Arabidopsis thaliana</i>
e-value for closest PDB Protein to SEQ ID NO: 288	4E-16
% Identity between SEQ ID NO: 288 and the closest protein from PDB	32
% Positives between SEQ ID NO: 288 and the closest protein from PDB	52
Number of Protein Hits in the VGDB to SEQ ID NO: 288	6
Number of Microorganisms having VGDB Hits to SEQ ID NO: 288	5
Microorganisms having VGDB Hits to SEQ ID NO: 288 <sup>1</sup>	[saur][nmen][bsub][efae][spne]
First predicted epitopic region of SEQ ID NO: 288: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 293 :NDLVSTICLATPAP-VAVLPAM, 1.195, 128->148
Second predicted epitopic region of SEQ ID NO: 288: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 294 :WADLVILAPAT-ADLIARVAAG, 1.175, 105->125
Third predicted epitopic region of SEQ ID NO: 288: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 295 :LLGQLLLDEIVTR, 1.163, 411->423

- 5           <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Eschericia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpxx* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

**FIGURE 253**

SEQ ID NO: 296

ATGAAAGTCATAGAAAGTGACACATCCTATACAATCTAAACAGTATATTA  
5 CAGAGGATGTTGCAATGGCATTTCGGATTTTTTCGATGGCATGCATAAAGGTCATG  
ACAAAGTCTTTGATATATTAAACGAAATAGCTGAGGCACGCAGTTTAAAAAAA  
GCGGTGATGACATTTGATCCGCATCCGTCTGTCGTGTTGAATCCTAAAAGAAAA  
CGAACAACGTATTTAACGCCACTTTCAGATAAAATCGAAAAAATTAGCCAACA  
TGATATTGATTATTGTATAGTGGTTAATTTTTTCATCTAGGTTTGCTAATGTGAGC  
10 GTAGAAGATTTTGTGAAAATTATATAATTAAAAATAATGTAAAAGAAGTCATT  
GCTGGTTTTGATTTTACTTTTGGTAAATTTGGAAAAGGTAATATGACTGTACTTC  
AAGAATATGATGCGTTTAATACGACAATTGTGAGTAAACAAGAAATTGAAAAT  
GAAAAAATTTCTACAACCTTCTATTCGTCAAGATTTAATCAATGGTGAGTTGCAA  
AAAGCGAATGATGCTTTAGGCTATATATATTCTATTAAAGGCACTGTAGTGCAA  
15 GGTGAAAAAAGGGGAAGAACTATTGGCTTCCCAACAGCTAACATTCAACCTAG  
TGATGATTATTTGTTACCTCGTAAAGGTGTTTATGCTGTTAGTATTGAAATCGGC  
ACTGAAAATAAATTATATCGAGGGGTAGCTAACATAGGTGTAAAGCCAACATT  
TCATGATCCTAACAAAGCAGAAGTTGTCATCGAAGTGAATATCTTTGACTTTGA  
GGATAATATTTATGGTGAACGAGTGACCGTGAATTGGCATCATTTCTTACGTCC  
20 TGAGATTAAATTTGATGGTATCGACCCATTAGTTAAACAAATGAACGATGATAA  
ATCGCGTGCTAAATATTTATTAGCAGTTGATTTTGGTGATGAAGTAGCTTATAA  
TATCTAG



**FIGURE 254**

SEQ ID NO: 297

          MKVIEVTHPIQSKQYITEDVAMAFGFFDGMHKGHDKVFDILNEIAEARS  
5 KAVMTFDPHPSVVLNPKRKRTTYLTPLSDKIEKISQHDIDYCIVVNFSSRFANVSVE  
DFVENYIIKNNVKEVIAGFDFTFGKFGKGNMTVLQEYDAFNTTIVSKQEIENEKIST  
TSIRQDLINGELQKANDALGYIYSIKGTVVQGEKRGRTIGFPTANIQPSDDYLLPRK  
GVYAVSIEIGTENKLYRGVANIGVKPTFHDPNKAEVVIEVNIFDFEDNIYGERVTVN  
WHHFLRPEIKFDGIDPLVKQMNDKSRACYLLAVDFGDEVAYNI

**FIGURE 255**

SEQ ID NO: 298

ATGAAAGTCATAGAAAGTGACACATCCTATACAATCTAAACAGTATATTA  
5 CAGAGGATGTTGCAATGGCATTTCGGATTTTTTCGATGGCATGCATAAAGGTCATG  
ACAAAGTCTTTGATATATTAAACGAAATAGCTGAGGCACGCAGTTTAAAAAAA  
GCGGTGATGACATTTGATCCGCATCCGTCTGTCGTGTTGAATCCTAAAAGAAAA  
CGAACAACGTATTTAACGCCACTTTCAGATAAAATCGAAAAAATTAGCCAACA  
TGATATTGATTATTGTATAGTGGTTAATTTTTTCATCTAGGTTTGCTAATGTGAGC  
10 GTAGAAGATTTTGTGAAAATTATATAATTAAAAATAATGTAAAAGAAGTCATT  
GCTGGTTTTGATTTTACTTTTGGTAAATTTGGAAAAGGTAATATGACTGTACTTC  
AAGAATATGATGCGTTTAATACGACAATTGTGAGTAAACAAGAAATTGAAAAT  
GAAAAAATTTCTACAACCTTCTATTCGTCAAGATTTAATCAATGGTGAGTTGCAA  
AAAGCGAATGATGCTTTAGGCTATATATATTCTATTAAAGGCACTGTAGTGCAA  
15 GGTGAAAAAAGGGGAAGAACTATTGGCTTCCCAACAGCTAACATTCAACCTAG  
TGATGATTATTTGTTACCTCGTAAAGGTGTTTATGCTGTTAGTATTGAAATCGGC  
ACTGAAAATAAATTATATCGAGGGGTAGCTAACATAGGTGTAAAGCCAACATT  
TCATGATCCTAACAAAGCAGAAGTTGTCATCGAAGTGAATATCTTTGACTTTGA  
GGATAATATTTATGGTGAACGAGTGACCGTGAATTGGCATCATTCTTACGTCC  
20 TGAGATTAAATTTGATGGTATCGACCCATTAGTTAAACAAATGAACGATGATAA  
ATCGCGTGCTAAATATTTATTAGCAGTTGATTTTGGTGATGAAGTAGCTTATAA  
TATCTAG

**FIGURE 256**

SEQ ID NO: 299

5      MKVIEVTHPIQSKQYITEDVAMAFGFFDGMHKGHDKVFDILNEIAEARS  
KAVMTFDPHPSVVLNPKRKRTTYLTPLSDKIEKISQHDIDYCIVVNFSSRFANVSVE  
DFVENYIIKNNVKEVIAGFDFTFGKFGKGNMTVLQEYDAFNTTIVSKQEIENEKIST  
TSIRQDLINGELQKANDALGYIYSIKGTVVQGEKRGRTIGFPTANIQPSDDYLLPRK  
GVYAVSIEIGTENKLYRGVANIGVKPTFHDPNKAEVVIEVNIFDFEDNIYGERVTVN  
WHHFLRPEIKFDGIDPLVKQMNDKSRKAYLLAVDFGDEVAYNI

**FIGURE 257**

SEQ ID NO: 300

Forward PCR Primer

5 GCGGCGGCCCATATGAAAGTCATAGAAGTGACAC

SEQ ID NO: 301

10

Reverse PCR Primer

GCGCGGATCCTTTTTCGATTTTATCTGAAAGTG

**FIGURE 258****TABLE 52 Properties of riboflavin kinase/FAD synthase from *S. aureus***

TABLE 52 -- riboflavin kinase/FAD synthase from <i>S. aureus</i> -- SEQ ID NO: 296-SEQ ID NO: 299	
Melting temperature (°C) of SEQ ID NO: 300 (forward PCR primer)	60
Restriction enzyme for SEQ ID NO: 300 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 301 (reverse PCR primer)	58
Restriction enzyme for SEQ ID NO: 301 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 296	972
Number of amino acid residues in SEQ ID NO: 297	323
Number of different nucleic acid residues between SEQ ID NO: 296 and SEQ ID NO: 298	0
Number of different amino acid residues between SEQ ID NO: 297 and SEQ ID NO: 299	0
Calculated molecular weight of SEQ ID NO: 297 polypeptide (kDa)	36.7
Calculated pI of SEQ ID NO: 297 polypeptide	5.6
Solubility of SEQ ID NO: 299 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching 100%
Solubility of SEQ ID NO: 299 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	No detectable expression
Amount of purified polypeptide having SEQ ID NO: 299, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	9.77
Amount of purified polypeptide having SEQ ID NO: 299 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	13.96
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. No interacting proteins were observed by using at least one of the methods described in those examples.	

**FIGURE 259****TABLE 53 Bioinformatic Analyses of riboflavin kinase/FAD synthase from *S. aureus***

TABLE 53 -- riboflavin kinase/FAD synthase from <i>S. aureus</i> -- SEQ ID NO: 296-SEQ ID NO: 299	
COG Category	coenzyme metabolism
COG ID Number	COG0196
Is SEQ ID NO: 297 classified as an essential gene?	Yes
Most closely related protein from PDB to SEQ ID NO: 297	None
Source organism for closest PDB protein to SEQ ID NO: 297	N/A
e-value for closest PDB Protein to SEQ ID NO: 297	N/A
% Identity between SEQ ID NO: 297 and the closest protein from PDB	N/A
% Positives between SEQ ID NO: 297 and the closest protein from PDB	N/A
Number of Protein Hits in the VGDB to SEQ ID NO: 297	11
Number of Microorganisms having VGDB Hits to SEQ ID NO: 297	10
Microorganisms having VGDB Hits to SEQ ID NO: 297 <sup>1</sup>	[saur][bsub][efae][ecoli][hinf][spne][paer][nmen][ctra][mgen]
First predicted epitopic region of SEQ ID NO: 297: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 302 :QHDIDYCVVNFS, 1.216, 87->99
Second predicted epitopic region of SEQ ID NO: 297: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 303 :PHPSVVLNP, 1.180, 59->67
Third predicted epitopic region of SEQ ID NO: 297: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 304 :AKYLLAVD, 1.157, 307->314

- <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Eschericia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpax* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

**FIGURE 260**

SEQ ID NO: 305

ATGAACCGAGTGCTGTACCCAGGCACCTTCGATCCCATCACCAAGGGTC  
5 ACGGCGATCTGATCGAACGTGCTTCACGGCTTTTCGACCATGTGATCATCGCGG  
TCGCCGCCAGCCCCAAGAAGAACCCCTGTTCAGCCTGGAACAGCGGGTTGCG  
CTGGCCCAGGAGGTCACCAAGCACCTGCCGAACGTCGAGGTGGTGGGCTTCTC  
CACCCTGCTGGCGCACTTCGTCAAGGAGCAGAAGGCGAATGTCTTCCTCCGCGG  
CCTGCGCGCGGTTTCCGACTTCGAGTACGAGTTCCAGCTGGCCAACATGAACCG  
10 CCAGCTCGCCCCGACGTGGAAAGCATGTTTCCTCACCCCGTCGGAGAAGTATTC  
CTTCATTTCTCGACGCTGGTCCGGGAAATCGCCGCTCTCGGCGGGGATATCAG  
CAAGTTCGTGCATCCGGCCGTGGCAGACGCCCTGGCGGAACGTTTCAAGCGCT  
GA

**FIGURE 261**

SEQ ID NO: 306

MNRVLYPGTFDPITKGHGDLIERASRLFDHVIIAVAASPKKNPLFSLEQRVAL  
5 AQEVTKHLPNVEVVGFSTLLAHFVKEQKANVFLRGLRAVSDFEYEFQLANMNRQL  
APDVESMFLTPSEKYSFISSTLVREIAALGGDISKFVHPAVADALAERFKR



**FIGURE 262**

SEQ ID NO: 307

ATGAACCGAGTGCTGTACCCAGGCACCTTCGATCCCATCACCAAGGGTC  
5 ACGGCGATCTGATCGAACGTGCTTCACGGCTTTTCGACCATGTGATCATCGCGG  
TCGCCGCCAGCCCCAAGAAGAACCCCCTGTTTCAGCCTGGAACAGCGGGTGGCG  
CTGGCCCAGGAGGTCACCAAGCACCTGCCGAACGTTCGAGGTGGTGGGCTTCTC  
CACCTGCTGGCGCACTTCGTCAAGGAGCAGAAGGCGAATGTCTTCCTCCGCGG  
CCTGCGCGCGGTTTCCGACTTCGAGTACGAGTTCAGCTGGCCAACATGAACCG  
10 CCAGCTCGCCCCGACGTGGAAAGCATGTTCCCTACCCCGTCGGAGAAGTATTC  
CTTCATTTCTCGACGCTGGTCCGGGAAATCGCCGCTCTCGGCGGGGATATCAG  
CAAGTTCGTGCATCCGGCCGTGGCAGACGCCCTGGCGGAACGTTTCAAGCGCT  
GA

**FIGURE 263**

SEQ ID NO: 308

MNRVLYPGTFDPITKGHGDLIERASRLFDHVIIA VAASPKKNPLFSLEQRVAL  
5 AQEVTKHLPNVEVVGFSTLLAHFVKEQKANVFLRGLRAVSDFEYEFQLANMNRQL  
APDVESMFLTPSEKYSFISSTLVREIAALGGDISKFVHPAVADALAERFKR

**FIGURE 264**

SEQ ID NO: 309

Forward PCR Primer

5 GCGGCGGCCCATATGAACCGAGTGCTGTACC

SEQ ID NO: 310

10

Reverse PCR Primer

GCGCGGATCCGCGCTTGAAACGTTCCGC

**FIGURE 265****TABLE 54 Properties of phosphopantetheine adenylyltransferase from *P. aeruginosa***

TABLE 54 -- phosphopantetheine adenylyltransferase from <i>P. aeruginosa</i> -- SEQ ID NO: 305-SEQ ID NO: 308	
Melting temperature (°C) of SEQ ID NO: 309 (forward PCR primer)	58
Restriction enzyme for SEQ ID NO: 309 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 310 (reverse PCR primer)	58
Restriction enzyme for SEQ ID NO: 310 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 305	480
Number of amino acid residues in SEQ ID NO: 306	159
Number of different nucleic acid residues between SEQ ID NO: 305 and SEQ ID NO: 307	0
Number of different amino acid residues between SEQ ID NO: 306 and SEQ ID NO: 308	0
Calculated molecular weight of SEQ ID NO: 306 polypeptide (kDa)	17.8
Calculated pI of SEQ ID NO: 306 polypeptide	9.6
Solubility of SEQ ID NO: 308 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching 100%
Solubility of SEQ ID NO: 308 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	Approaching 100%
Amount of purified polypeptide having SEQ ID NO: 308, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	29.8
Amount of purified selmet labeled polypeptide having SEQ ID NO: 308, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	46.0
Amount of purified polypeptide having SEQ ID NO: 308 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	7.5
Amount of purified selmet labeled polypeptide having SEQ ID NO: 308 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	93.0
Results of protein interaction study described in EXAMPLE 11, EXAMPLE 12, EXAMPLE 13 and EXAMPLE 14. No interacting proteins were observed by using at least one of the methods described in those examples.	

## FIGURE 266

**TABLE 55 Bioinformatic Analyses of phosphopantetheine adenylyltransferase from *P. aeruginosa***

TABLE 55 -- phosphopantetheine adenylyltransferase from <i>P. aeruginosa</i> -- SEQ ID NO: 305-SEQ ID NO: 308	
COG Category	coenzyme metabolism
COG ID Number	COG0669
Is SEQ ID NO: 306 classified as an essential gene?	Yes
Most closely related protein from PDB to SEQ ID NO: 306	phosphopantetheine adenylyltransferase (1qjc)
Source organism for closest PDB protein to SEQ ID NO: 306	<i>Escherichia coli</i>
e-value for closest PDB Protein to SEQ ID NO: 306	3E-51
% Identity between SEQ ID NO: 306 and the closest protein from PDB	61
% Positives between SEQ ID NO: 306 and the closest protein from PDB	90
Number of Protein Hits in the VGDB to SEQ ID NO: 306	10
Number of Microorganisms having VGDB Hits to SEQ ID NO: 306	9
Microorganisms having VGDB Hits to SEQ ID NO: 306 <sup>1</sup>	[paer][ecoli][saur][bsub][hinf][spne][hpyl][nmen][bbur]
First predicted epitopic region of SEQ ID NO: 306: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 311 :ERASRLFDHVIIA VAAS, 1.186, 22->38
Second predicted epitopic region of SEQ ID NO: 306: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 312 :SKFVHPAVADALAE, 1.165, 142->155
Third predicted epitopic region of SEQ ID NO: 306: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 313 :KNPLFSLEQRVALAQ-EVTKHLPNVEVVGFSTLLAHFVKE, 1.156, 41->79

- 5           <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Escherichia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlamydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpax* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.

**FIGURE 267**

SEQ ID NO: 314

ATGAAAGCTTCTCTGCTGAAAAAGCTGGATGTCCTCAGCGATCGCTACG  
5 AAGAACTGACGGCGCTGCTCGGCGACGCCGAGGTGATCAGTGACCAGACCCGC  
TTCCGCGCCTATTCCCGCGAGTACGCCGAGGTCGAACCGGTGATCCTGGCGTTC  
CGCGACTACCGCAAGGTGCAGGCCGACCTCGAGGGCGCCCAGGCGTTGCTCAA  
GGACAGCGACCCGGAGTTGCGCGACCTCGCCGAGGAGGAGGTGCGCCGAAGCGC  
GCGGCCGCCTCGCCGCCCTCGGCGACAGCCTGCAGCGCATGCTGCTGCCGAAG  
10 GATCCCAACGACAGCCGCAACGTGTTCTGAGATCCGTGCCGGCACCGGTGG  
CGACGAGGCGGGCGATCTTCTCCGGCGACCTGTTCCGCATGTATTCGCGCTACGC  
CGAGCGCCAGGGCTGGCGGATCGAGACGCTGTCCGAGAACGAGGGCGAGCAC  
GGTGGCTACAAGGAAGTGATTGCCCAGGTCGAGGGCGACAACGTCTACGCCAA  
GCTCAAGTTCGAGTCCGGCGCGCACCGCGTGCAGCGGGTGCCGGAACCGAAT  
15 CCCAGGGCCGGATCCACACTTCCGCCTGCACCGTCGCGGTGCTGCCGGAGCCG  
GACGAGCAGGCAGCGATCGAGATCAACCCGGCCGACCTGCGGGTGACACCTA  
CCGTTCTCCGGTGCCGGCGGCCAGCACGTCAACAAGACCGACTCGGCGGTGC  
GCATCACCCACATTCCCAGCGGCATCGTGGTCGAGTGCCAGGAAGAGCGCTCG  
CAGCACAAGAACCGCGCCAAGGCCATGGCCTGGCTGGCGGCCAAGCTCAACGA  
20 CCAGCAGCAGGCCGCGGCGCAGCAGGCGATCGCCAGCACGCGCAAGCTGCTGG  
TGGGCTCGGGCGACCGCTCGGAGCGCATCCGTACCTACAACCTCCCGCAAGGG  
CGGGTCACCGACCATCGCATCAACCTCACCTGTACTCCCTGGGCGAGGTGATG  
GAGGGCGCGGTGGAACAGGTGATCGAGCCGCTGCTGCAGGAATACCAGGCCGA  
TCAACTGGCGGCCCTGGGCGACTGA

**FIGURE 268**

SEQ ID NO: 315

          MKASLLKKLDVLSDRYEELTALLGDAEVISDQTRFRAYSREYAEVEPVILAF  
5  RDYRKVQADLEGAQALLKDSDPDLRLAEEVVAEARGRLAALGDSLQRMMLPKD  
      PNDSRNVFLEIRAGTGGDEAAIFSGDLFRMYSRYAERQGWRIETLSENEGEHGGYK  
      EVIARVEGDNVYAKLKFESGAHRVQRPETESQGRIHTSACTVAVLPEPDEQAAIEI  
      NPADLRVDTYRSSGAGGQHVNKTDHAVRITHIPSGIVVECQEERSQHKNRAKAMA  
      WLAACLNDQQQAAAQQAIASRKLLVGSGDRSERIRTYNFPQGRVTDHRINLTLY  
10 SLGEVMEGAVEQVIEPLLQEYQADQLAALGD

**FIGURE 269**

SEQ ID NO: 316

ATGAAAGCTTCTCTGCTGAAAAAGCTGGATGTCCTCAGCGATCGCTACG  
5 AAGAACTGACGGCGCTGCTCGGCGACGCCGAGGTGATCAGTGACCAGACCCGC  
TTCCGCGCCTATTCCCGCGAGTACGCCGAGGTCGAACCGTTGATCCTGGAGTTC  
CGCGACTACCGCAAGGTGCAGGCCGACCTCGAGGGCGCCCAGGCGTTGCTCAA  
GGACAGCGACCCGGAGTTGCGCGACCTCGCCGAGGAGGAGGTGCGCCGAAGCGC  
GCGGCCGCCTCGCCGCCCTCGGCGACAGCCTGCAGCGCATGCTGCTGCCGAAG  
10 GATCCCAACGACAGCCGCAACGTGTTCTGAGATCCGTGCCGGCACCGGTGG  
CGACGAGGCGGCGATCTTCTCCGGCGACCTGTTCCGCATGTATTCGCGCTACGC  
CGAGCGCCAGGGCTGGCGGATCGAGACGCTGTTCGGAGAACGAGGGCGAGCAC  
GGTGGCTACAAGGAAGTGATTGCCCGGGTCGAGGGCGACAACGTCTACGCCAA  
GCTCAAGTTCGAGTCCGGCGCGCACCGCGTGCAGCGGGTGCCGGAAACCGAAT  
15 CCCAGGGCCGGATCCACACTTCCGCCTGCACCGTCGCGGTGCTGCCGGAGCCG  
GACGAGCAGGCAGCGATCGAGATCAACCCGGCCGACCTGCGGGTGGACACCTA  
CCGTTCTCCGGTGCCGGCGGCCAGCACGTCAACAAGACCGACTCGGCGGTGC  
GCATCACCCACATTCCCAGCGGCATCGTGGTCGAGTGCCAGGAAGAGCGCTCG  
CAGCACAAGAACCGCGCCAAGGCCATGGCCTGGCTGGCGGCCAAGCTCAACGA  
20 CCAGCAGCAGGCCGCGGCGCAGCAGGCGATCGCCAGCACGCGCAAGCTGCTGG  
TGGGCTCGGGCGTCCGCTCGGAGCGCATCCGTACCTACAACTTCCCGCAAGGGC  
GGGTCACCGACCATCGCATCAACCTCACCTGTACTCCCTGGGCGAGGTGATGG  
AGGGCGCGGTGGAACAGGTGATCGAGCCGCTGCTGCAGGAATACCAGGCCGAT  
CAACTGGCGGCCCTGGGCGACTGA



**FIGURE 270**

SEQ ID NO: 317

          MKASLLKKLDVLSDRYEELTALLGDAEVISDQTRFRAYSREYAEVEPLILEF  
5  RDYRKVQADLEGAQALLKDSDPELRDLAEEVVAEARGRLAALGDSLQRMMLPKD  
      PNDSRNVFLEIRAGTGGDEAAIFSGDLFRMYSRYAERQGWRIETLSENEGEHGGYK  
      EVIARVEGDNVYAKLKFESGAHRVQRPETESQGRIHTSACTVAVLPEPDEQAAIEI  
      NPADLRVDTYRSSGAGGQHVNKTDSAVRITHIPSGIVVECQEERSQHKNRAKAMA  
      WLAACLNDQQQAAAQQAIASRKLLVGSGVRSERIRTYNFPQGRVTDHRINLTLY  
10 SLGEVMEGAVEQVIEPLLQEYQADQLAALGD

**FIGURE 271**

SEQ ID NO: 318

Forward PCR Primer

5 GCGGCGGCCCATATGAAAGCTTCTCTGCTGAAAAAG

SEQ ID NO: 319

10

Reverse PCR Primer

GCGCAGATCTGTCGCCCAGGGCCGCC

**FIGURE 272****TABLE 56 Properties of peptide chain release factor 1 from *P. aeruginosa***

TABLE 56 -- peptide chain release factor 1 from <i>P. aeruginosa</i> -- SEQ ID NO: 314-SEQ ID NO: 317	
Melting temperature (°C) of SEQ ID NO: 318 (forward PCR primer)	66
Restriction enzyme for SEQ ID NO: 318 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 319 (reverse PCR primer)	60
Restriction enzyme for SEQ ID NO: 319 (reverse PCR primer)	BglII
Number of nucleic acid residues in SEQ ID NO: 314	1083
Number of amino acid residues in SEQ ID NO: 315	360
Number of different nucleic acid residues between SEQ ID NO: 314 and SEQ ID NO: 316	3
Number of different amino acid residues between SEQ ID NO: 315 and SEQ ID NO: 317	3
Calculated molecular weight of SEQ ID NO: 315 polypeptide (kDa)	40.041
Calculated pI of SEQ ID NO: 315 polypeptide	4.8
Solubility of SEQ ID NO: 317 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approaching 100%
Solubility of SEQ ID NO: 317 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	No detectable expression
Amount of purified polypeptide having SEQ ID NO: 317, prepared and purified as described in the Exemplification (mg/L of culture). The polypeptide so expressed and purified is His tagged and has the additional amino acid residues of SEQ ID NO: 1 at the N-terminus as described in EXAMPLE 6.	4.83
Amount of purified polypeptide having SEQ ID NO: 317 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	12.70

## FIGURE 273

TABLE 57 Bioinformatic Analyses of peptide chain release factor 1 from *P.**aeruginosa*

TABLE 57 -- peptide chain release factor 1 from <i>P. aeruginosa</i> -- SEQ ID NO: 314-SEQ ID NO: 317	
COG Category	translation, ribosomal structure and biogenesis
COG ID Number	COG0216
Is SEQ ID NO: 315 classified as an essential gene?	Yes
Most closely related protein from PDB to SEQ ID NO: 315	Release factor 2, (1gqe)
Source organism for closest PDB protein to SEQ ID NO: 315	<i>Escherichia coli</i>
e-value for closest PDB Protein to SEQ ID NO: 315	1E-57
% Identity between SEQ ID NO: 315 and the closest protein from PDB	38
% Positives between SEQ ID NO: 315 and the closest protein from PDB	57
Number of Protein Hits in the VGDB to SEQ ID NO: 315	25
Number of Microorganisms having VGDB Hits to SEQ ID NO: 315	13
Microorganisms having VGDB Hits to SEQ ID NO: 315 <sup>1</sup>	[paer][hinf][ecoli][nmen][bsub][saur][spne][ctra][hpyl][bbur][rpxx][mgen][efae]
First predicted epitopic region of SEQ ID NO: 315: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 320 : TSACTVAVLPE, 1.209, 200->210
Second predicted epitopic region of SEQ ID NO: 315: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 321 : YAEVEPVILAFR, 1.164, 42->53
Third predicted epitopic region of SEQ ID NO: 315: amino acid sequence, rank score, amino acid residue numbers	SEQ ID NO: 322 :SAVRITHIPSGIVVECQ, 1.153, 244->260

- 5           <sup>1</sup>Organisms are abbreviated as follows: *ecoli* = *Escherichia coli*; *hpyl* = *Helicobacter pylori*; *paer* = *Pseudomonas aeruginosa*; *ctra* = *Chlaydia trachomatis*; *hinf* = *Haemophilus influenzae*; *nmen* = *Neisseria meningitidis*; *rpxx* = *Rickettsia prowazekii*; *bbur* = *Borrelia burgdorferi*; *bsub* = *Bacillus subtilis*; *staph* = *Staphylococcus aureus*; *spne* = *Streptococcus pneumoniae*; *mgen* = *Mycoplasma genitalium*; *efae* = *Enterococcus faecalis*.